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A COMPARATIVE STUDY OF THE GROWTH OF SKIN AND HUMAN SKIN TUMORS ON THE CHORIO-ALLANTOIC MEMBRANE OF THE EMBRYONATED CHICKEN EGGS

BY

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PREFACE

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INTRODUCTION

Since long scientists have been engaged in investigating the possibilities to maintain viable tissues outside the original milieu. It would be of great importance, especially in cancer research, to find a suitable culture medium taking into consideration the characteristics of the tissue. Moreover, the cultivation of the tissue outside the original host yields the advantage that various functions of the transplanted tissue can be observed under better controlled circumstances.

Unfortunately, the transplantation of tissues from one specimen to another seems to be very difficult. Different culture methods have been used, the most important of which are, from one point of view, the *in vitro* method and, from another, the transplantation methods with different animals. The most important among the latter are the so-called cheek pouch method in hamsters used by different workers, the anterior chamber method proposed by G r e e n e (31), preventive treatment of animals by X-rays and cortisone before transplantation introduced by T o o l a n (100), and the method of cultivating the tissue in the embryonated chicken eggs reported by R o u s and M u r p h y (76). This method, still applicable and of current interest, will be discussed in more detail in the present study.

In 1911, R o u s and M u r p h y (76) reported that after injection of the suspension prepared from a Rous chicken sarcoma into the chick embryo, a tumor formation, entirely corresponding to the original tumor tissue, could be established, especially on the chorio-allantoic membrane (CAM) of the chicken egg. A year later, M u r p h y (57) found that heterologous tissue, such as rat and mouse tumors, also grew well on the CAM. These findings brought new possibilities in the field of homo- and heterotransplantation. In the rapidly growing membranes of a chicken embryo there were growth-promoting factors for other tissues, too. In addition, the fact that the chick embryo was hardly able to mobilize specific reactions to foreign tissue (78, 30, 71, 80-84) made the embryonated chicken egg suitable for transplantation experiments.

Attempts have also been made to use the chicken egg for the cultivation of human tissues, but no systematic comparisons of the growth of normal and malignant human tissue in the egg have been performed. In the present study, an attempt has been made to elucidate these problems and suggest further explanations concerning the possibilities to use embryonated chicken eggs in transplantation studies in general.

SURVEY OF THE LITERATURE

In virology, the use of embryonated chicken eggs has gained a central position among different culture methods. The embryologists have likewise made much use of the embryonated chicken egg and specially of the chorio-allantoic membrane in their studies, because embryonic tissues of different animals, such as those of hen, mouse and rat, have been found to grow and develop rather well on this membrane. On the contrary, attempts to cultivate adult normal human and animal tissues have been relatively rare and most of them have been undertaken as minor additional or completing studies.

The embryonated chicken egg offers extremely good living conditions for avian tumors, though mouse and rat tumors also grow well in it. The tumor tissue has been transplanted to the chicken egg either directly on the CAM or intraembryonically, intravenously and in the yolk, amniotic and allantoic sacs. With the aid of these methods, successful transplantation through several passages has been possible. Similar statement cannot, unfortunately, be given concerning experiments with human tumors. In spite of attempts with various methods, the embryonated chicken egg has not fulfilled the great expectations as culture medium. However, experiments on this field are scanty, and larger material is needed before final evaluation can be made on the application of the embryonated chicken egg for the transplantation of human tissue.

Chorio-Allantoic Membrane (CAM) Method

In egg cultures the CAM method is mostly used. It has been developed through the following phases. The original purpose of Rous and Murphy (76) was to transplant the tissue to the embryo itself. Embryonated hen eggs 7-8 days old were used. A rectangular piece was cut out from the shell, the shell membrane was turned away with sterile forceps, and the embryo with its membranes became visible. Tumor mass was injected through the CAM into the embryo. The piece of shell was replaced with the aid of paraffin. Ten days later the egg was opened for the study of the tumor tissue. However, Rous and Murphy (76) already reported at the end of their first paper that the results were

mainly taken from tumors growing on the CAM. During the injection, part of the tissue fastened on the CAM and started to grow in it. The tumor tissue growing on the CAM was found and studied much more easily than that growing in the embryo. Their next paper (63) gives the impression that they injected a part of the tumor tissue onto the CAM on purpose, and in later experiments only this membrane was used (57—62). Burnet (6) developed this method further into the so-called "false air sac" technique, in which, before breaking the egg membrane, a hole is drilled through the shell into the air space, and the CAM is dropped loose from the shell membrane. Thereby placing the inoculum on the membrane becomes much easier. The "false air sac" technique has been accepted into general use.

Several papers deal with the structure of the CAM (22, 24, 37, 11, 16, 73). From the fifth day on, the fusion of allantoic membrane and chorion begins, and after 10—12 days the CAM is complete. Danchakoff (11) found that the blood capillaries penetrated the ectodermal layer of the CAM on the 8th day; by the 13th to the 15th day they had extended on the membrane as a large capillary network. At that time a piece placed upon the membrane is under good nutritional conditions. It is mainly on the basis of these findings that transplantation has generally been performed on the 8th—9th days. There has been a general effort to use fairly young membranes in order to lengthen the short growth period of the tissue on the CAM. Minoura (56), when transplanting the homologous sexual gland tissue to the CAM, used eggs 2—16 days old, but found that grafts transplanted to eggs 8—11 days old produced the greatest changes in the embryos. He was of the opinion that the blood circulation of the membranes younger than 5 days was too undeveloped, and that in the embryos older than 2 weeks possibly a resistance to foreign tissues has been developed and that such embryos are thereby enabled to destroy the grafts. It has been proved in virology that different viruses need membranes of certain age for the best possible growth results (3, 89). In Rous sarcoma experiments Oker-Blom and Strandström (69) showed that the most rapid growth occurred on the membranes aged 11 days. The final size of the tumor was the same whether transplanted onto the young or onto the old CAM. The long incubation in poorer environments was apparently compensated by better conditions during the short incubation time.

In addition to good nutritional conditions, there is another important factor favoring the growth of the inocula on the membrane; the chicken embryo has no general defense mechanism against foreign tissues, which fully developed animals have (78, 30, 71).

It has been shown that the CAM with its fine structure easily responds to all kinds of stimuli. These reactions have been observed by almost every worker studying the CAM transplantation method. Macroscopically, flesh-colored thickenings and edematic areas appear around the site of transplantation. In microscopical examinations, *i. a.*, proliferation in the ectoderm and endoderm, edema, and stratification in the mesoderm as well as so-called epithelial pearls have been found. Hemorrhages and cytoplastic inclusions have been established to be due to viruses and viral tumors (3, 50), but similar changes have also been produced by some nonspecific substances, such as horse serum (9), broth and physiologic saline solution (90), as well as by strong evaporation (25), cooling (21) or reactions occurring in the intact membrane with age (16).

Tissue Transplanted to the CAM

Homologous Embryonic Tissue. — Soon after Rous and Murphy (76) embryologists began to transplant the embryonic tissue to the CAM, and almost every tissue of the hen at the embryonic stage has been studied. Only few of these studies will be mentioned in this connection. Kiyono and Sueyasu (52) and Dantschakoff (14) were probably the first to conduct large experiments in this field. The former showed that tissues with simple functions, *e. g.*, cartilage, skin and connective tissue grew more readily than those of complex functions (kidney, liver).

Dantschakoff (14) inoculated several embryonic tissues on the CAM. Her most important studies concerned, however, erythropoietic problems (12, 13). Thus she, in a way, carried on experiments on the lines of Murphy (57—62). The largest studies on embryonic tissues on the CAM were performed by Willier (103—107), who paid attention to the development of transplanted embryonic glands on the membrane and to their influence upon the growing embryo. Murray (64) transplanted lateral grafts from the region forming the posterior limb and showed that, *i. a.*, skin tissues grew well. This paper supports for its part the studies of other scientists (38, 52).

Embryonic tissue has also been transplanted on the CAM for other purposes. Sandström and co-workers (80—84) performed a series of experiments transplanting the kidney tissues of hens and ducks of various ages and cartilage of ducks to elucidate the problems of tissue specificity reactions on the CAM. Hancox (33) studied bone formation by transplanting pieces of the frontal bone of the chicken embryo to the CAM.

A good and pithy review on the embryologic studies was given by Rawles (72) in 1952.

Heterologous Embryonic Tissue. — Murphy (57) already transplanted successfully embryonic tissue of the rat to the CAM without describing in details the growth of tissue on it. In 1927, Hiraiwa (36) transplanted anterior, middle and posterior thirds of rat embryos aged 11 days on the CAM 8—9 days old. He found that the epidermis, cartilage and bone tissue grew and differentiated best. "The epidermis, hair follicles, cartilages and bone differentiated to a degree comparable with that of normal rat embryos of corresponding age, nasal sacs and mesonephros showed considerable differentiation, nervous and endodermal structures exhibited practically no capacity for independent differentiation." In his opinion, this could possibly be explained by the fact that the tissue with lower metabolic functions was better fit for the new environment than the tissue with active cell functions. Nicholas and Rudnick (65) performed later a larger experiment testing "the proliferation and differentiation capacity of various embryonic rat tissues in a closely graded series of stages ranging from six to eleven days transplanted to the CAM." They found that the tissues on the CAM maximally reached the same size as the controls, though the majority remained smaller.

Waterman (102) compared the behavior of embryonic rabbit and rat tissues in the kidneys of the adult rat, in the kidneys and omental bursa of the rabbit and on the CAM. He found that the differentiation and growth of the tissues was poor and abnormal in all laboratory animals. None of the tissues grew longer than 6—8 days, and cartilage and bone tissues were the most vigorous. In his opinion, "the chorioallantois grafts showed an intermediate condition between homoiotransplants on the one hand and heterotransplants on the other".

As regards experiments on the human embryonic tissue, Dantschakoff and Gagarin (15) transplanted human embryonic hearts aged 3—5 weeks to the CAM. The heart lived well and even beat during the 10 days it lay on the membrane. Accordingly, the heart was capable to utilize the nutriment offered by the chick embryo.

Human chorionic and amniotic membranes were also transplanted on the CAM chiefly for virus research (28). As these membranes are very thin they can utilize the nutriment through the plasmatic metabolism so that the transfer to a foreign environment produced no major disturbances. They readily adhered to the CAM and lived well on it.

Homologous Adult Tissue. — Little is known about the behavior of the normal adult tissue on the CAM. Oakley (66) compared the behavior

of the liver tissue of hen, duck and rat embryos with the corresponding tissue of the adult hen and showed that adult hen liver did not generally survive well on the CAM. Sandström (80—82) reported that the kidney tissue of the adult hen was unable to rebuild its excreting mechanism on the CAM.

Heterologous Adult Tissue. — Heterologous tissue has been transplanted on the membrane by several workers. Schwartz (87) transplanted intact and macerated kidney tissue of the rat, aged from 15 days to adult. The still undeveloped kidney tissue aged 15 days grew and differentiated on the membrane, but the older tissue necrosed in direct correlation with increasing age of the tissue. Fully developed tissue formed, however, an exception being able to persist "because of its high degree of physical differentiation and organization". In his opinion, the probable causes of necrosis were as follows:

1) Poor formation of blood vessels during the incorporation period.

2) The accumulation of small round cells around the transplant whereby they formed mechanical hindrances to the development of the transplant.

3) Probably the lack of normal function, *i. e.*, the kidney tissue could not live longer on the membrane because it did not receive the specific stimulus (uric acid) for its normal function.

All transplants began to die after the CAM had reached the age of 20 days. This was explained by Schwartz (87) and also by some others (99) as due to the fact that the CAM dried up at the time of hatching.

Being aware of the successful cultivation of the heterologous tissue, Goodpasture, Douglas and Anderson (26) transplanted human skin to the CAM in form of Thiersch-grafts in 1938. They found that the skin lived on the membrane fairly well and reported, among other things, the following results. "In successful experiments the epithelium of the chorio-allantois fuses with that of the graft, the collagen fibers of the corium interlace with those of the membrane after the separation or disappearance of the ectodermal layer, and the blood vessels of the chick anastomose, and unite by intervening pools of extravasated blood, with those of the graft. This vascular communication between the two tissues is largely responsible for the nourishment of the graft by affording a plasmatic circulation. Gradually there is a partial revascularization of the graft by an ingrowth of blood vessels from the chick membrane." ... "Occasionally regrafts upon a second egg have succeeded and thus prolonged the vitality of the graft to 14 days."

The same research group (27—29), as well as Blank, Coriell and Scott (4), later used skin on the CAM for virological studies. The

human conjunctiva has also been cultivated on the CAM (44, 23, 51, 88). It showed good viability and in some cases it could be transplanted even through five passages. Thus it afforded good material for viral studies and in this way the alterations caused by trachoma viruses could be followed in the human conjunctiva.

Rubovits and Abrams (77) transplanted human ovarian and endometrial tissue to the CAM. Both grew fairly well and on the 4th—5th day after the transplantation the blood vessels penetrated into the grafts. The less specified tissue showed greater viability on the CAM than the fully specialized one. According to them, transplantation to the next passages ought to take place on the 4th—5th day of growth, before the capillary network has developed between the host and the graft. No mention of attempts at passaging can, however, be found in their paper.

Avian Tumors. — A long period of time elapsed after the preliminary works by Rous and Murphy (76, 57—62) before any attention was paid to avian tumor grafts with the CAM method. In 1939, Rothbard and Herman (75) made an attempt to transplant avian tumor produced by benzpyrenes to the CAM, but they did not succeed in it. Since 1940, Duran-Reynals and co-workers (17—20, 55) have studied the behavior of avian tumors in birds at different stages of development and also transplanted these tissues to the CAM in this connection. In 1942, Pierce (70) transplanted suspension taken, *i. a.*, from birds suffering avian leukemia to the CAM. The suspension contained spleen cells, leukocytes and plasma and produced leukemic changes in the embryos. Six years later, Karnofsky and co-workers (45) reported on the transplantation of several tissues, *e. g.*, avian tumors to the CAM. In the same year Campbell (7—8) transplanted both spontaneously growing and chemically induced avian and mammalian tumors to the CAM, but high infection and mortality rates were disadvantages in his work. In his opinion "slow growing tumour usually gives disappointingly small growths whilst normal adult tissues fail to grow and be absorbed". Lastly, Oker-Blom (67) transplanted different avian tumors to the chick embryo by using various methods. His experiments confirmed the earlier concepts that the CAM is a good culture medium for avian tumors.

Mammalian Tumors. — Jensen rat sarcoma was obviously the first heterologous tissue transplanted to the CAM by Murphy in 1912 (57). It grew on the membrane at least through 4 passages retaining its typical characteristics, though the tissue was looser forming a loose network here and there. Mitoses occurred more frequently in the tumors growing on the membrane than in the originals. In connection with these

tissues, other tumors were also transplanted successfully to the CAM, *e. g.*, Ehrlich mouse sarcoma and mouse chondroma, mammary carcinoma of mouse and Flexner Jobling adenocarcinoma of rat.

Stevenson (92—94) enlarged the field of these studies transplanting several mouse and rat tumors to the CAM. All tumors showed great vitality and several passages could be made. Macroscopically they were of the same size as the tumors proliferating in mice and rats for a similar period. Microscopically there was marked proliferation. The tumor cells inwandered from the transplant to the mesoderm of the membrane, the sarcoma cells one by one, the carcinoma cells in groups. The former seemed to be somewhat larger in the membrane than in the host animal, which according to Stevenson (92) was due to the lower pressure in the new environment. Mitoses occurred in abundance. As a rule, the characteristic features of the tumors remained on the membrane and no changes as to the rapidity of growth took place. On the contrary to Murphy (57—62), he found that the transplantation of adult hen spleen and bone marrow simultaneously with the tumor tissue produced no hindrances to the tumor growth.

The CAM has proved to be a suitable culture medium for the Walker rat tumor 256, the rat sarcoma 1239 and the Brown-Pearce rabbit carcinoma (86). The histologic features of the two latter on the membrane were similar to those in the original host. The Walker rat tumor 256 formed an exception, probably due to the fact that the tumor on the CAM reshaped its original carcinoma structure. Schechtman, Cohen and Berkowitz (85) also transplanted Brown-Pearce rabbit carcinoma to the CAM. Growth was successful in 50 per cent of the cases and the tumor could be transplanted through 8 passages. No increase or decrease occurred in the number of successful transplants through the passages. When several tumor sections were transplanted to the CAM simultaneously, the take percentage increased up to 75.2. Mostly the original implantation tissue resulted in necrosis, and fresh tumor cell accumulations developed in the mesoderm of the CAM. In these groups necrosis of the centrum was not found to be similar to that in tumors transplanted to rabbits.

Jacoby, McDonald and Woodhouse (43) made efforts to transplant a mouse sarcoma induced by dibenzanthracene to the CAM and thus form better controlled growth conditions, but the results were poor. In spite of occasional slight growth, the number of successful transplants and the size of the graft did not fulfill the expectations. This work confirms the statement of other writers (75, 7) that chemically induced tumors are mostly unable to grow on the CAM.

In recent years, Karnofsky and co-workers (45—47) have been studying the growth of different tumors in embryonated chicken eggs. They transplanted 27 different mouse tumors to the CAM and showed that each one of them maintained its characteristic histologic features. Many tumors were transplanted at least through 3 passages, some even oftener, *e. g.*, a mouse sarcoma 180, which survived on the CAM for a year. As to the growth capacity, these were grouped into six classes on the basis of their growth in the host and the CAM.

Human Tumors. — Murphy (57) made an attempt to transplant human sarcoma, but it did not grow so well as mouse and rat tumors. He assigned this to the fact that it took some time before the tissue could be transplanted from a human being to the CAM. Stevenson (94) also transplanted 9 different human tumors and tumor metastases to the CAM of 350 eggs. They were 5 mammary carcinomas, 1 mammary carcinoma metastasis, 1 squamous cell carcinoma of the leg, 1 carcinoma of testicles and 1 chorio epithelioma of the uterus. He hardly established any growth. "There is little hope of being able to maintain a tumor of slow growth indefinitely to the chick. Consequently the cultivation of human tumors in the chick embryo for more than a short period of time does not seem likely to be realized." In Stevenson's (94) opinion, tumors of rapid growth were more successful on the membrane than those growing slowly, but the former showed signs of exhaustion earlier, because the tumors were incapable to utilize the foreign proteids of the chick embryo.

Hurst and his associates (41) agreed with Stevenson (94), as their results from human tumors were not corresponding to those obtained from animal tumors by earlier workers. In most cases, malignant cells were less viable than the normal connective and fat tissue around them. They attempted to transplant 6 carcinomas of breast, 5 secondary from carcinoma of stomach, 1 secondary from carcinoma of lip, 1 secondary melanoma of liver, 1 meningioma and some benign tumors of man and rabbit as well as normal adult tissue to the CAM of 467 hen and duck eggs. The duck eggs were used because of their 5 weeks' hatching time, and the tissue could thus remain on the membrane longer. However, the experiments with the latter did not fulfil the expectations because of the high mortality of the eggs. This was explained as due to the fact that incubation conditions were not good enough for duck eggs.

Sommers, Sullivan and Warren (91) transplanted 59 human carcinomas on the CAM of 375 eggs and divided the results into positive, questionable and negative cases. By positive results they meant persistence or growth. In questionable cases some possibly living

cells could be identified, while in negative cases no living tumor cells were found. A hundred and forty-nine eggs lived until the checking time of the tumors. Of them 36 were positive, 26 questionable and the rest negative. Degenerative changes were in the foreground and mitoses were seldom met. The transplantation of sarcoma tissues succeeded best, as out of the 47 eggs used 13 grafts were positive and 6 questionable. In total, 146 transplants were performed from adenocarcinoma and carcinoma simplex tissues. Thirty-two of them succeeded, while 33 were considered questionable. Epidermoid carcinoma was found in 15 of the 99 eggs used, besides 3 doubtful cases. Thus the successful transplant yield was about one-quarter of the eggs with sarcomas, one-fifth with glandular carcinomas and about one-seventh with epidermoid carcinomas. S o m m e r s and co-workers (91) were of the opinion that equally good results were obtained by the CAM method as by the other cultivation methods used in their laboratory.

K a r n o f s k y and his research group (47) had the largest material in this field. In a paper on the transplantation of tumors to the chick embryo in general they reported to have transplanted, *i. a.*, 71 human tumors to the CAM during 3 years. The tumors were as follows: 21 cases of *lymphogranulomatosis maligna* tissue, 28 lymphosarcomas and lymphatic leukemias, and 22 miscellaneous tumors including 5 pulmonary carcinomas, 4 mammary carcinomas and 2 melanomas. As a rule, the tumors showed poor viability and mitoses were established in only few cases. Attempts at transplantation to other eggs were unsuccessful. They did not find, either, that human tissue would cause major changes in the embryo apart from *lymphogranulomatosis maligna* tissue which produced general edema in 60 per cent of the embryos. Edema was most remarkable at the 3rd day and disappeared generally at the 10th day. Only a small part of the embryos died of edema, but those survived were later of smaller size than the control animals. The active substance causing edema survived in the refrigerator for 2 months. Edema was thought to be induced by a chemical agent present at some part of tissue in *lymphogranulomatosis maligna*. Lymphogranulomatosis tissue proper and lymphosarcoma and leukemia tissues did not grow on the CAM. On the other hand, P i e r c e (70) reported on the successful though short-time cultivation of leukocytes of leukemic blood on the membrane, while normal white blood cells did not survive on it. Leukocytes of leukemic blood were also met with in the liver and perivascular tissue of the embryo, but they all were degenerated. No changes in the embryos were found, not even after hatching.

During recent years, two investigations have been published differing from the earlier through their better results. In 1952, K a u f m a n and co-workers (48) reported in a short abstract on the cultivation of 26 human tumors on the CAM. Out of these, 6 tumors were only transplanted through one passage and they lived well for 11 days. Out of the 20 other tumors, which principally were grafted with the intention of prolonging survival by serial transplants, 15 were maintained during the primary explantation. Of these, 5 survived one retransplantation (the second passage) and 2 survived two retransplantations (the third passage).

In 1956, in a more detailed description (49), they reported that the first-mentioned 6 tumors were 4 thyroid carcinomas, one adenocarcinoma of the breast and one squamous cell carcinoma of the lung. All explants survived the incubation period and a great number of cells in each explant appeared healthy. In addition, some normal thyroid tissue was grafted with one of the tumors and it was also in a state of good preservation.

"Seven of the 20 tumors were sarcomas. These sarcomas were implanted upon 137 eggs which lived. The tumors survived on 77 of these eggs. Six of the seven strains of sarcoma lived. These six were transplanted to 40 eggs and grew on 13. Three of the six strains survived the second transplant. These, in turn, were transplanted to 10 eggs and one of the three sarcomas remained viable. This tumor survived for a total of 27 days, but after the third graft showed extensive degeneration.

Two astrocytomas and one glioblastoma survived the primary transplant. These were planted on 42 eggs and 24 showed tumor. One astrocytoma survived a second and third transfer. This particular tumor was planted on 6 eggs for the second transfer and all showed tumor. The third transfer was to 2 eggs which survived and both showed tumor. Thus, one astrocytoma survived 3 transfers for a total of 22 days.

Three of the four breast carcinomas survived the first transfer, although all subsequent transplanting failed. Twenty-three of 70 eggs which were planted and which lived contained tumor. However, for one of the tumors, 15 eggs were planted and all 15 showed tumor at the end of a 5-day period.

One thyroid carcinoma survived the initial transplant. Of 26 eggs which were planted, 15 showed tumor but none of the explants survived the second transfer.

One neurilemoma was planted on 4 eggs that lived. Two eggs showed tumor. These two fragments were cut up and regrafted on 4 eggs, 2 of which again showed tumor. This represented a survival of 15 days.

One of two melanocarcinomas survived the first graft. This one was

planted on 12 eggs, 6 of which contained tumor but none survived the second graft. The other was planted on 25 eggs and none of the fragments survived.

One renal cell carcinoma was planted on 4 eggs but no positive takes were obtained."

In another study, published in 1954, D a g g, K a r n o f s k y, T o o l a n and R o d d y (10) used 3 types of tumors as transplantation material, which were produced by injecting suspended tumor mince of 3 human carcinomas into rats and hamsters treated by cortisone and X-rays and maintained in them for several months (100). The sarcoma type HSl grew on the CAM well through 8 passages and retained its histologic appearance. The tumor could also be cultivated in the yolk sac. The epithelial carcinoma type HEp3 was transplanted through 6 passages. After the third membranous passage it was also cultivated in the yolk sac. The tumor maintained its characteristic features on the CAM and metastased into the liver and kidneys. The third tumor tissue was an embryonic muscle sarcoma type, which was cultivated on the CAM through 3 passages. In the rat the tumor was formed from small dark staining cells, which could also be seen well preserved on the CAM. Two of the former tumor tissues were retransplanted to rats and they grew further without differing from the original, in the rat grown tumor in any way. In a previous work, K a r n o f s k y and his co-workers (45) found that nitrogen mustard HN2 had certain chemotherapeutic effects on the mouse sarcoma 180, and it was therefore injected into the yolk sac of the eggs in which HEp3 and HSl tumor tissues grew. The macroscopic size of the tumors was smaller, irregularity in cell nuclei and marked decrease in cellularity was observed, but the influence of HN2 was markedly smaller than in the sarcoma 180.

In this laboratory, human tissues have also been cultivated as a preliminary experiment (68). Tissue of the following 39 human tumors was transplanted: 4 naevi, 2 melanomas, 1 spinocellular carcinoma and 2 basocellular carcinomas of the skin, 4 spinocellular carcinomas of the lip, 1 spinocellular carcinoma of the penis, 3 carcinomas of the stomach, 1 carcinoma of the uterus, 1 carcinoma of the lung, 1 fibrosarcoma, 1 sarcoma of the leg, and 3 laryngeal papillomas.

In addition, normal human skin, placental tissue and skin of the chick embryo were cultivated. Of the heterologous tissues, naevus tissue and normal skin grew best. In some cases, spinocellular carcinoma could be transplanted to the second passage. As a rule, the growth of the tissues was poor, but larger series were needed before final criticism could be given on the suitability of the CAM for a culture medium of the human tissue.

Yolk Sac Method

Especially, Taylor and his group (95—98) made use of this method. They obtained the best results in cultivating DBA and CH3 mouse tumors. They also succeeded in cultivating several other mouse and rat tumors in the yolk sac, and according to them, one of the advantages of this method was that the tumors grew freely in the yolk sac cavity not being under the mechanical pressure of membranes. Later this technique has also been used in other laboratories (34, 35, 101, 1), but it has not become general. *E. g.*, according to Heilman and Bittner (35) the method is not suitable for routine use because of the high mortality of eggs (72 per cent) and the small number of takes. In spite of several efforts (95, 34, 101) the cultivation of human tumors in the yolk sac has been unsuccessful.

Amniotic and Allantoic Sac Methods

Thiersch (99) using the method described by Burnet (6) injected leukemic tissue of man and mouse into the amniotic cavity of the chick embryo and showed by convincing material that no leukemia developed in chick embryos or in hatched chickens. In addition, different animal tissues (53) have been cultivated in the amniotic and allantoic cavities, though these methods have not come into general use for transplantation purposes.

Intravenous Cultivation Method

Thiersch (99) used this method by injecting blood of human leukemia into the umbilical vein of the chick embryo and found in 5 cases out of 12 that 6 months after hatching the chickens showed signs of "big bone disease", which, according to him, referred to a leukemic reaction. Bender, Friedgood and Lee (2) succeeded in cultivating the CH57 mouse sarcoma in the liver and kidneys of the chick embryo by injecting this tissue into the allantoic vein. They also injected human tumor suspension intravenously, and of 9 tumors used 4, *viz.*, 2 neuroblastomas, 1 hemangioblastoma and 1 cerebral metastasis of pulmonary carcinoma, were found in the liver of the embryos. Poor growth as well as the small number of takes was in their opinion due to the elapse of time before the tumor tissue could be moved from man to the egg. Murphy (57) had earlier come to the same conclusion with his CAM cultivations. The intravenous cultivation method has been used relatively little, probably because of the difficult technique. The

mortality of embryos is also high, according to B e n d e r and co-workers (2) 50 per cent. The table compiled by T h i e r s c h (99) gives the impression that his results were not better in this respect. Even this sets certain limitations to the use of the method.

I n t r a e m b r y o n i c C u l t i v a t i o n M e t h o d

By transplanting tissue to the embryo itself it has been possible to cultivate both embryologic, adult normal and tumor tissues in the chick embryo (32, 72, 53). Especially when inoculating whole limb buds the method has its advantages. In addition, tissue can be cultivated in the same individual for longer periods by allowing the embryo to hatch. The method has, however, its disadvantages. *E. g.*, it is difficult to find the transplanted tissue, and thus the method can be used only for the tissues growing relatively well. The method is so far rather young and is not specially favored.

Summary

Several tissues have been successfully cultivated in the embryonated chicken egg by using different methods. The CAM technique seems to be the best, at least in major experiments. In spite of its fairly common use, there seem to be no more detailed investigations on the significance of the age of the CAM for the growth of mammalian tissue. On the contrary, several papers mention the reactions of the membrane to different stimuli. The evaluation of these reactions must be performed very carefully.

Of homologous embryonic tissues, at least simple tissues of ectodermal origin, such as skin, have grown well and the similar fact has been established in cultivating certain heterologous normal tissue. Of tumor tissues, especially fowl, rat and mouse tumors have grown well in the egg, and it has even been possible to divide some of them into groups on the basis of their viability. Human tumors, on the other hand, have shown poorer growth. So far, these studies have been rather rare and the results somewhat contradictory. As a rule, numerous different malignant tumors have been cultivated, but comparative studies between the normal and malignant forms of the same tissue have not been performed.

PRESENT INVESTIGATION

Plan for the Study

As pointed out in the survey of the literature, the cultivation of different normal and malignant animal tissues on the CAM has been successful. The cultivation experiments with human tissue have, on the contrary, yielded poorer results. However, human skin tissue has been cultivated on the membrane relatively seldom and it might be possible that the cultivation experiments with a large material could bring with a tissue specimen, a tumor tissue especially, the growth characteristics and capacity of which would be so suitable for this purpose that it could continually be cultivated on the membrane. This has been a stimulating thought when undertaking the present study.

As far as is known, systematic comparisons on the living functions of normal and malignant forms of the same tissue on the CAM have not been carried out previously. The main purpose of the present study was therefore to transplant normal and malignant human skin tissue to the CAM of the embryonated chicken egg, to compare the growth phenomena of these tissues, and, in addition, to study the possible changes produced by the tissue in the chicken embryo.

The skin tissue was selected for the experiments, as skin has proved to be one of the best viable tissues on the CAM (26, 27, 29, 4). Besides, it was more easily available than other types of human tissue both at various stages of development and in different malignant forms. It was but natural that the homologous skin tissue was selected for control material, as it corresponding to other test tissue was likewise growing relatively well on the membrane.

The different phases of the study were divided as follows.

1) First, an attempt was made to determine at which stage of development the CAM offered the best living conditions for the skin tissue grafts. Then using the most suitable membrane,

2) To observe the viability of the homologous skin tissue of different stages of development on the CAM,

3) To observe the viability of the normal human skin of different stages of development on the CAM,

4) To perform comparisons between the viability of the homologous and heterologous normal tissue,

5) To observe the viability of benign human tumors of the skin on the CAM,

6) To observe the viability of malignant human skin tumors on the CAM,

7) To perform comparisons between the normal and neoplastic tissues and to observe the possible changes produced by these in the embryonated chicken eggs,

8) To transplant both normal and malignant tissue of the human skin on the same membrane and to observe the possible mutual influences of the tissues,

9) To try to transplant the above-mentioned tissues through several egg passages and, if possible, to find a tissue type that could continually be transplanted on the membrane.

Material

All the 1060 embryonated Leghorn eggs used in the present study were from the same hatchery. The following tissues were transplanted to the CAM.

A. Homologous tissue
   1) Skin of the chick embryo
   2) Skin of the chicken
   3) Skin of the adult hen

B. Heterologous tissue
   Normal tissue
   1) Embryonic tissue of the human skin
   2) Adult tissue of the human skin
   Tumors
   1) Benign tumors of the human skin
   2) Malignant tumors of the human skin

C. Normal and malignant human skin tissue side by side on the same membrane

Homologous Tissue. — In total, skin of the chick embryo was transplanted to 60, chicken skin grafts to 60, and skin of the adult hen to 52 eggs.

Heterologous Tissue. — The heterologous tissue contained solely human skin tissue, which was transplanted both in normal condition and in different skin tumors.

The normal skin contained both embryonic skin, which was obtained from 4 embryos 2—5 months of age and transplanted to 105 eggs in total, and adult human skin which was transplanted to 291 eggs.

The skin tumors were obtained both from malignant and benign types. The benign skin tumors were as follows: 1 keloid, 4 fibromas, 7 naevi, 4 verrucae of the sole, and 5 leukoplakia tissues. Tissue from these tumors was transplanted to 158 eggs in total. The malignant skin tumors were taken from 30 basocellular, 15 spinocellular, 3 baso-spinocellular, and 3 anaplastic skin carcinomas as well as from 3 mammary carcinomas adherent to the skin. In total, malignant tissue was transplanted to 309 eggs. In addition, tissue from 3 basocellular and 3 spinocellular carcinomas was transplanted to 25 eggs simultaneously with the normal skin tissue.

Method

Before transplantation the embryonated chicken eggs were incubated in a hatching machine at 37° C, where the eggs were turned twice a day. The relative moisture of the air was 70 per cent. After transplantation the eggs were preserved in an incubator, where the temperature and the relative moisture of the air were the same as in the hatching machine. Except a few modifications, the inoculation method used was the so-called "false air sac" technique by Burnet (6). The eggs were candled with a lamp specially constructed for the purpose, and the position of the air space and the largest membranous vessels were noted and outlined with a pencil. First, the general method was used in which a window of 1 sq.cm was sawn from the shell with a carborundum disc attached to a dental drill. With this method, however, the CAM easily adhered to the shell and the shell membrane, obviously because of frictional heat. Therefore the eggs were later cut with a blunt-edged instrument constructed from a surgical knife by grinding. A small groove was made on the egg shell and a piece about 1 sq.cm in size was removed by lifting the shell.

The rough breakage of eggs was equally slight in both methods, but reactions on the membrane and mortality of eggs were greater during the incubation period in the former method. Moreover, it was easier to discover the damages on the membrane of the egg in which the shell was removed with the instrument, as the damaged point was in the middle of the membrane, and not in the edges as in the sawing method. A qualified worker could open the eggs as rapidly with the instrument as with the drill. — In the present study all the eggs were opened with the blunt-edged instrument.

After removing the piece of shell a small hole was made with a sharp awl through the shell and the shell membrane of the air sac. A drop of sterile saline was applied to the shell membrane to prevent petechia formation, which occasionally occurred when the CAM loosened from the egg shell. Through the drop a hole was pierced gently with a blunt instrument through the shell membrane when the water penetrated between the CAM and the shell membrane. By sucking the air sac the CAM was dropped from the shell membrane which was now removed from the new cavity, and the graft could be placed on the CAM as wanted, usually at the blood vessel. An attempt was made to cut all the grafts about $4 \times 5 \times 0.5-1$ mm in size. A disadvantage difficult to eliminate was that some grafts tended to roll when placed on the membrane. This rolling not only caused bleeding on the membrane when efforts were made to straighten the graft but, in all probability, also prevented and slowered incorporation. Rolling and wrinkling were the more common the more the skin contained elastic tissue. Adult human skin was most difficult to deal with, while jelly-like chick embryo skin and human embryo skin were easier to place. After several attempts, it proved to be best to place the graft evenly on the CAM by cutting it extremely thin with even thinner edges. By grasping the edges of the graft with fine forceps so that the corium side opposite to the membrane was turned convex the graft could be placed on the membrane fairly well and without notable lesions.

After placing the graft on the membrane the window in the shell was sealed with transparent Scotch tape so that the graft could be observed daily by means of a magnifying glass. Every other day a specimen was taken from each series by cutting 1—2 tissue grafts and the surrounding CAM for histologic examination. Each chick embryo was dissected and tissue specimens were taken from the liver, spleen and lungs for the examination of possible changes.

The sterility control was also performed from each dead embryo on a blood agar plate. The dead embryos could be divided into two groups. The first group contained embryos with no signs of infection. It is probable that factors, such as the opening of the egg shell, cold, the breakage of the membrane and perhaps even the influence of the tissues on the embryo caused the death of the embryo. The trauma mortality was 10 per cent, in total. The infected embryos, 2.4 per cent in total, formed the second group.

All precautions were taken in order to avoid contamination. Before the inoculation the tissues were kept for a short period in sterile saline containing 500 units of penicillin and 50 mg of streptomycin per ml.

The egg shell was wiped with 60—70 per cent ethyl alcohol and the instruments were sterilized by boiling them before use, and kept in 60 per cent alcohol during the experiments. The egg lay on cotton wool soaked in alcohol.

Histologic Examination

A piece of the CAM with skin grafts was placed on a thin piece of paper to prevent wrinkling. The piece of paper had, however, to be removed before embedding in paraffin. The tissue specimen was fixed in Bouin's fluid, dehydrated in increasing ethanol-butanol series, embedded in paraffin, cut into sections of 5 μ and stained with hematoxylin and van Gieson's stain.

To evaluate the growth capacity of the grafts, the original tissue was studied microscopically paying special attention to possible inflammatory changes. The quality and amount of inflammation as well as the occurrence of necrosis were noted in this connection.

In malignant specimens, the grade of differentiation was studied also histologically. In all original tissue specimens, special features of the tissue were studied, such as skin glands and hair follicles and pigmentary cells of the naevi. The features occurring in the grafts were compared with those of the original tissue specimens.

Evaluation of the Results

Each specimen was examined with the naked eye, with magnifying glass and with microscope. The results of the grafting were divided into following groups on the basis of the histologic picture.

a) Good results refer to grafts showing good viability, which was comparable with the control material, *i. e.*, with the fresh tissue grafted.

b) Fairly good results refer to grafts in which part of the tissue was still viable, but in which necrosis also occurred. Accordingly, these grafts did not live well on the membrane.

c) Questionable results refer to the grafts in which the tissue resulted in necrosis, but in which here and there in the edge of the graft or deep in the membrane some living cells were seen that could be assumed to belong to the original graft.

d) Poor results refer to entirely necrosed grafts.

The first and the last groups were easily distinguished, while it was much more difficult to classify the tissues in the other groups. Had the dividing criterion been only "good", "poor" and "medium", the classi-

fication would have been too rough. In the microscopical evaluation the main attention was paid to the graft in its entirety, but graft cells living on the mesoderm of the membrane were also noted, if possible. The macroscopical examination made the evaluation easier, but because the graft often sank inside the membrane, macroscopical evaluation was not always possible.

Optimal Transplantation Age of the CAM

When determining the optimal age of the CAM, skin of the chick embryo and chicken was selected for test tissues, as it was easily available, grew well on the membrane, and because its basic structure was fairly similar to the human skin. Thus we also obtained material that enabled us later to compare the growth of homologous and human skin tissues as well as the reactions they produced. In each group, 10 grafts were inoculated (Table 1, p. 28).

Five-Day-Old Membrane. — In this series more dead transplantation grafts were met with than in the others. It is evident that a membrane of this age is too young and undeveloped for transplantation purposes. At the start the tissues probably lived on plasmatic circulation, but as early as after six days increasing degeneration occurred. On the 5th day, many grafts still swam loose on the membrane because of delayed fixation. Three grafts disappeared and could not be found, though the shell was totally removed and the embryo with its membranes was examined under the water, when as a rule disappeared grafts were easily found. They had obviously sunk in the embryo through the thin membrane, or slid away from the membrane and dried on the egg shell, or been resorbed because of poor nutrition. One embryo was infected by *Staphylococcus albus*. Traumatic deaths of the eggs were fairly rare. It might be mentioned, however, that during the manipulation many eggs were spoiled because of the easy breakage of the CAM.

Seven-Day-Old Membrane. — The seven-day-old membrane offers more favorable circumstances, which was clearly established by more successful grafts. Delayed fixation of the grafts, however, still occurred. One of the grafts disappeared. As a new phenomenon, a vesicle formation occurred under the CAM which will, however, be discussed in more detail later on.

Nine-Day-Old Membrane. — It seemed evident that the 9-day-old membrane was the most suitable for the transplantation of skin tissue, as all transplanted grafts were viable. Follicle formation occurred in 4 cases. Some bleedings occurred on the membrane, but they did not

TABLE 1

The Skin of the Chick Embryo and Chicken on the CAM

SERIES	Skin of 10-Day-Old Chicken Embryo on 5—15-Day-Old CAM						Skin of 4-Day-Old Chicken on 5—15-Day-Old CAM					
	XVII	XXI	XX	XIV	XIX	XIII	VIII	XII	IX	XI	VII	X
Age of the eggs (days)	5	7	9	11	13	15	5	7	9	11	13	15
Number of specimens	1	1	1	1	1	1	1	1	1	1	1	1
Number of eggs	10	10	10	10	10	10	10	10	10	10	10	10
Bleeding on the CAM (cases)	—	—	2	3	4	7	—	—	—	3	4	10
Trauma	2	2	1	4	—	3	—	1	1	1	2	—
Infection	—	—	—	—	—	—	1	1	—	—	—	—
Good	—	6	7	4	5	1	—	5	6	3	5	6
Fairly good	1	1	2	1	4	5	1	1	3	4	2	3
Poor	6	1	—	1	—	1	6	1	—	2	1	1
Graft cannot be found	1	—	—	—	1	—	2	1	—	—	—	—
Graft loose on the membrane	4	—	—	—	—	—	5	2	—	—	—	—
Vesicle formation on the membrane	—	5	4	—	—	—	—	—	—	—	—	—

interfere with the growth of the grafts. On younger membranes I did not see any hemorrhages.

Eleven-Day-Old Membrane. — The number of viable grafts was similar to that in the previous series their condition on the membrane being, however, slightly poorer. Bleedings tended to be abundant and interfered with the growth of the grafts by forming tar-like shells around them.

Thirteen-Day-Old Membrane. — Bleeding tendency still increased. Fixation of the grafts was extremely good and rapid, but in spite of this there seemed to occur no great positive changes for the growth. It seemed likely that the otherwise favorable circumstances could not compensate the shortened period of time to live on the membrane.

Fifteen-Day-Old Membrane. — In this series, bleeding occurred in each membrane and interfered with the growth of the grafts by forming black tar-like rings around them. The results were not so good as those of the 9-day-old series. Taking also into consideration the shortened period of maintenance, it is obvious that the 15-day-old membrane is not suitable for grafting.

Summary

The 5—7-day-old CAM is still too unripe for grafting. Grafts did not adhere rapidly enough to membranes younger than 8 days, which was probably due to their poor vascularization. Moreover, grafts tended to sink in younger membranes more often. This phenomenon showed no evident influence upon the viability of grafts, but it made the macroscopical evaluation difficult. Bleedings, on the other hand, occurred on too old membranes disturbing, to some extent, the growth of grafts. Previous workers (65) have also paid attention to the unfavorable influence of bleeding.

On the basis of these experiments, it seemed likely that the 9-day-old CAM would be the most suitable for transplantation. Therefore in later experiments, eggs of this age were used, though 10—11-day-old eggs had to be used occasionally.

HOMOLOGOUS SKIN TISSUE ON THE CAM

Skin of the Chicken Embryo

The macroscopic and microscopic evaluations were based on the grafts taken from 10-day-old chick embryos and transplanted to the membranes of 9—11 days of age (Table 1, p. 28).

Macroscopic Features. — Down feathers could not be detected in the skin of the 10-day chick embryos by the naked eye. The skin was jelly-like and difficult to flay. This succeeded best in water in which the skin could clearly be seen as a thin pellicle. The piece of skin was placed on the CAM without difficulties owing to its jelly-like composition, and it adhered during the first 24 hours. In most cases the graft remained convex on the CAM. It was flesh-colored and could thus easily be distinguished from the membrane. Due to membranous bleeding, the color turned brownish and with the age jelly-likeness disappeared while the piece hardened considerably. On the 4th—5th day, 2—4 mm long down feathers could be observed in the grafts. On the 10th—11th day, they had grown up to about 8 mm. In the controls, the down feathers were about 10 mm long and grew thicker than in the grafts. This referred to inhibition in the down growth. Five embryos died of trauma, but no infections occurred.

Microscopic Features. — Out of 15 grafts, 11 showed good and 3 fairly good growth. One graft resulted in necrosis. After the first 24 hours the grafts adhered firmly to the membrane. The ectodermal layer of the membrane was swollen, and its cells seemed to be partially degenerated. The dermal papillae of the graft were primitive. They, together with the vascular layer, were relatively abundant in blood cells (Fig. 1). After 5 days the dermal papillae had developed considerably. The ectoderm of the membrane had mostly disappeared, and in the preparations in which it still survived the ectodermal cells were degenerated (Fig. 2a). At the end of the test series, the 9th day, various components of the graft were found evenly expanded inside the mesoderm of the membrane. Compared with the control preparations, major differences could not be found between different tissue layers, except shorter and thinner down feather rudiments. The corium was looser in the grafts and no demarcation line between the graft and the membrane was found. Epidermis tended to wrinkle in the folds with keratin accumulations (Fig. 3).

Reactions on the Membrane. — As mentioned previously, the CAM responded extremely sensitively to all stimuli. Two days after the transplantation of the graft, light-gray veil-like cloudiness in the form of an irregular star was observed on the membrane. Later it took a faster fusiform shape surrounding wall-like the graft. Another common phenomenon was the accumulation of blood vessels around the graft, as reported by, *e. g.*, Danchakoff (11) and Willier (103). As a rule, the graft was placed on the blood vessel, which then enlarged. Gradually other blood vessels also penetrated into the graft. It almost seemed as if the graft had drawn them to it.

The membranous hypertrophy around the graft, as seen in the microscope, proved to be due to the hypertrophy of all membranous layers where the mesodermal hypertrophy was prevailing. The ectodermal and endodermal hypertrophies varied in each case. They occurred mostly in the folds of the membrane, or in the sites where the graft and the ectoderm became attached. The mesodermal hypertrophy was pronounced and very common. Contrary to the ectodermal and endodermal hypertrophies, it decreased with the increasing age of the membrane. At the site of the membrane where the graft lay there occurred degeneration and disappearance of the ectoderm, as mentioned previously. In some cases, small islands of the ectoderm survived, and it seemed likely that these were the cause of the development of the so-called epithelial pearls.

The cellular reactions of the membrane were rather slight in this series. Only in the cases in which the graft was necrosed inflammatory cell accumulation could be noted in the close neighborhood of the grafts. Typical of this series was a large vesicle formation 2 cm in diameter on the endodermal layer, exactly underneath the graft. This phenomenon was already shortly mentioned in the preliminary study (68). Previously, Murray (64) and Karnofsky with co-workers (45) noticed a similar vesicle formation when transplanting rudiments of 2-day-old chick embryos to the 8-day-old CAM.

Almost every grafted tissue caused some reactions, but a large sac formation was found only in the series in which homologous embryonic tissue was used. Accordingly, it is possible that embryonic tissue influenced the development of the vesicle formation. It occurred only in young membranes, 7—9 days of age. Mostly, the vesicle became adherent to the endoderm of the membrane with a thin stem. It contained clear mucous substance, and its wall consisted of 3 layers, as did the membrane itself. The loose middle layer was encircled on each side by

membranes formed from flat cells, the microscopical structure of the wall thus closely resembling the normal CAM.

Reactions in the Embryo. — No marked changes were found in the embryos. In the 5 cases, in which the embryos were allowed to hatch, the chickens grew normally without differing from the controls in any way. The microscopic preparations from the lungs, liver and spleen were equally similar to those of the controls.

Skin of the Chicken

The skin grafts were taken from 4-day-old chickens. For uniformity, also in this series only the grafts transplanted to 9—11-day-old membranes were taken into consideration in the evaluation of the results (Table 1, p. 28).

Macroscopic Features. — The graft became adherent to the membrane on the 1st—2nd day. The piece lay convex on the CAM. No marked enlargement in the size of the graft could be observed during the 9 days when the graft was on the membrane. On the contrary, the impression was gained as if the graft had shrunk, which was probably partly due to the fact that the margins of the skin graft turned inside forming a more convex formation on the CAM. The skin tissue seemed to have been growing on the membrane, though the growth was slower than in the controls. This became most evident when comparing the down growth between the grafts and the control animals. When the skin grafts were flayed from the back of the chicken, the down was ca. 1 cm of length and was not dense. On the 10th—11th day, the down feathers of the graft were 1.5 cm long, but 0.5 cm shorter than those of the controls. The most remarkable difference was, however, in the density of the down, because the number of down feathers in the grafts did not seem to have increased being half as scarce as that of the controls.

Microscopic Features. — Out of the 18 grafts, 9 showed good and 7 fairly good viability, while 2 grafts resulted in necrosis. In most preparations, degeneration and disappearance of the ectoderm of the CAM was to be noted as soon as the 2nd day. On the 4th—5th day, no uniform ectoderm could be found any more, but the mesoderm of the membrane penetrated into the graft and its fibrocytes and thin capillaries of blood vessels interlaced between the connective tissue fibers of the corium. The maximal period of the graft on the CAM was 9 days and during this time proliferation was noted both in its epidermis and dermal papillae. The graft was considerably thickened and no demarcation line could be

noted between the graft and the membrane. The comparison of the graft with the control preparation revealed that the epidermal layer of the graft corresponded to the control tissue, while differences occurred in other layers. The connective tissue of the dermis was, on the contrary, considerably loose forming the main part of the graft. Occasional hyalinization was observed in the connective tissue. The down feather papillae were developed, though they never reached the same size as the control material. That was also true for the small muscles surrounding the down feather rudiments.

Reactions on the Membrane. — In this series, too, the membranes reacted to the graft as previously by forming white veil-like cloudiness around the graft on the 2nd—3rd inoculation day. After 4—5 days the veil formation hypertrophized into a wall-like disc around the graft. As compared with the previous series, the disc was greater, probably because the graft was harder than the previous embryonic tissue. No vesicle formation was seen, although the disc could hypertrophize so that it grew above the margins of the graft, whereby the graft sank into the CAM. Sinking was the greater, the thicker the piece was and the more it was wrinkled. However, the piece never sank into the CAM entirely, at least the center always remained on the surface. It was stated earlier that sinking was influenced by the age of the membrane.

Microscopically, no great difference was found qualitatively in the reactions of the CAM as compared with previous experiments. The ectoderm disappeared from under the graft as usual and hypertrophized around the graft. Mesodermal and endodermal changes were likewise similar to those in previous series. Inflammatory cells were found only around dead grafts. They did not occur in viable grafts.

Reactions in the Embryo. — No notable changes occurred in the embryos.

Skin of the Adult Hen

The tissue grafts from 3-year-old hen were transplanted to 53 eggs in total. Before flaying, the back skin of the hen was picked clean from feathers and wiped with 30 per cent alcohol. The skin was flayed along the subcutis, the graft was cut into standard pieces and soaked in penicillin-streptomycin solution as previously (Table 2, p. 34).

TABLE 2

Skin of the Adult Hen on CAM

Number of Series	Hö L – HöLI
Number of specimens	1
Number of eggs	52
Trauma	3
Infection	1
Good	15
Fairly good	24
Poor	9

Macroscopic Features. — During 4—5 days the majority of the grafts sank hypertrophized into the CAM forming a sac inside it. Possibly because of wrinkling, the size of the piece became smaller on the membrane; accordingly, proper macroscopic growth was not visible. New feathers did not grow. During the experiment, 3 embryos died without any signs of infection. One embryo was infected by a mold, which occurred occasionally but did not, in general, lead to the death of the embryos.

Microscopic Features. — On the remaining 48 membranes, 39 grafts in total were viable, 15 of which showed good and 24 fairly good viability. Nine grafts resulted in necrosis. Wrinkling and rolling of grafts, frequent in almost every preparation, had obviously unfavorable effects upon the viability. Most grafts were surrounded by the mesoderm of the membrane and on the 4th—5th day the small blood vessels of the membrane grew into the dermal part of the graft. When comparing the successful preparations with the controls no great differences were seen except the appearance of inflammatory cells. The epidermal layer of the graft showed probably greater keratinization and the grafts were often very folded, but no other changes could be found.

Reactions on the Membrane. — Inflammatory cell accumulations were also observed abundantly around the viable grafts (Fig. 4). In this series, the reason for the occurrence of inflammatory cells must have been some other than before, because the round inflammatory cells were accumulated only around the dead tissue. The age of the graft tissue may have played a certain role in the reaction. The other membranous reactions, *i. e.*, the disappearance of the ectoderm on the site of the graft and the membranous hypertrophies were, on the contrary, entirely similar to those in the previous experiments.

Reactions in the Embryo. — The spleen was slightly enlarged and redder than usual in some embryos, while other changes were not seen. Five chickens which were allowed to hatch and live after hatching looked quite normal.

Summary

The homologous skin tissue grew well on the CAM as a rule. The embryonic skin tissue showed the best and the adult chick skin the poorest viability. The tendency to wrinkle certainly played a role in the poor viability of the old skin tissue. This tendency did not appear in the young tissue. In embryonic and chicken skin tissue grafts, notable down feather growth was seen and mitoses were most numerous in young tissues, above all in the epidermis and around dermal papillae. Mitoses were also met with in adult skin tissue grafts as evidence of the growth.

The CAM responded to every inoculation irrespective of the age of the tissue as follows. Hypertrophies appeared both in the ectoderm, mesoderm and endoderm. One to three days after the inoculation, the ectoderm disappeared from the site where the graft was exposed and the remnants resembled so-called epithelial pearls. Besides these general reactions, a few phenomena possibly in connection with the quality of the graft were observed. These were the vesicle formation on the inner wall of the membrane, which was met with only when transplanting the embryonic skin, and the considerably abundant accumulation of inflammatory cells around adult skin grafts in the membrane. It seemed likely that the inoculations produced no changes in the embryo itself.

HETEROLOGOUS SKIN TISSUE ON THE CAM

Normal Human Skin

Goodpasture and co-workers (26) were probably the first to transplant human skin on the membrane. They used pieces of the same thickness as Thiersch grafts and gave a detailed report on the behavior of human skin on the membrane. The viability of skin was good and it could be used as testing material in various virologic and bacteriologic experiments (27, 29, 4). The experiments in the present study were mainly performed for comparative purposes and, accordingly, little attention was paid to histologic characteristics. The material included both embryonic and adult human skin.

Embryonic Human Skin

As far as is known, embryonic tissue has been cultivated on the membrane only once so far (15). Thus it was interesting to study the viability of the embryonic skin on the CAM. The skin grafts were taken from 5 embryos obtained from the Women's Clinic of the University of Helsinki, in connection with legal abortions. The age of the embryos varied from 2—5 months. The grafts were implanted about 3—4 hours after the operations. Meanwhile the embryos were stored in the refrigerator at 4° C. The skin tissue was flayed along the subcutis, so the grafts were 0.5—1 mm thick (Table 3).

TABLE 3

Human Embryonic Skin at Various Stages of Development on the 9-11-Day-Old CAM

Number of Series	Hu XVIII	Hu XVII	Hu XIX	Hu XX	Total
Age of Embryonic Skin (Months)	2	3	4	5	
Number of specimens	1	1	1	1	4
Number of eggs ..	30	10	20	10	70
Trauma	2	2	—	2	6
Infection	—	1	—	—	1
Good	12	2	14	5	33
Fairly good	11	4	3	2	20
Poor	5	1	3	1	10

Two-Month-Old Human Embryonic Skin

Macroscopic Features. — The skin tissue of this age was still rather jelly-like, and easily adhered to the membrane within 1—2 days. Most of the grafts remained on the membrane throughout the inoculation period, though a few grafts sank into the hypertrophized membrane resulting in fusiform formations. The maximum time the graft could be maintained on the membrane was 10 days, and during that time it thickened considerably developing a brownish or gray formation on or inside the membrane, resembling in form and shape a half of the pea. Simultaneously, however, the area of the grafts diminished. Therefore the thickening of the graft could not be due to the growth of the graft solely, but additional factors, such as the swelling of the piece and the rolling of its margins had to be taken into consideration. The latter phenomenon was, however, slight in grafting embryonic skin.

Microscopic Features. — In total 30 grafts were cultivated. Two embryos died of trauma, so 28 grafts were available for microscopic evaluation. Twelve of these grew on the membrane so well that the tissues were comparable to the controls. Eleven grafts showed fairly good viability, and 5 were entirely necrosed. The cells of the dermal layer of the graft very much resembled the mesodermal cells of the membrane, and it would have been difficult to draw the line between the graft and the membrane, had not the epithelial pearls survived in the ectoderm. Moreover, abundant vascular bed had developed in the dermal layer, which for its part, made the determination of the line easier. As soon as 1—2 days after the inoculation the blood vessels were filled with chick blood cells. After 9 days the dermal layer was thickened twice as large as its original size because of the multiplication of cells and the edema. Growth also occurred in the epidermal layer, and the mitoses were relatively frequent in the *stratum germinativum* and around the embryonic hair follicles (Fig. 5 a).

Three-Month-Old Human Embryonic Skin

The majority of the grafts in this series (like those of 5 months of age) was retransplanted to new eggs. Therefore the number of first passage grafts for evaluation was smaller than that in other series.

Macroscopic Features. — Three-month-old skin tissue did not differ much from the former, so the grafts were almost similar though somewhat faster, and adhered well to the membrane. Probably owing to small membranous bleeding, the grafts became slightly brownish and took on a convex round shape on the membrane. During the experiment, 3

embryos died, one of infection. The causative organism was *Micrococcus albus*.

Microscopic Features. — Of the 7 grafts, 2 showed good, 4 fairly good and 1 poor viability. Microscopically, the grafts resembled the tissue used in the former series. The dermal layer was most similar to that in the former series having thickened about 2—3 times from its original size. This was obviously caused by the multiplication of cells and by edema occurring in the grafts. No demarcation line was found between the graft and the membrane (Fig. 6 a). The epidermal layer was more developed and the *stratum germinativum*, *stratum intermedium* and *peridermis* could be discerned on it, the lines of the layers not being, however, so clear as in the control preparations. In some preparations a cellular layer formed from dead cells was seen on the periderm after 6—8 days, obviously the first elements of the *stratum corneum* layer.

Four-Month-Old Human Embryonic Skin

Macroscopic Features. — The tissue was clearly tougher and resembled more the adult skin tissue than that in the previous series, but the grafts lay well on the membrane. They were transparently white or brownish in color and tar-like hematoma was occasionally seen in the margins. The majority of grafts remained on the membrane throughout the inoculation period. The thickening and the corresponding reduction of the area of the graft were probably somewhat slighter than in two former series. None of the eggs died of trauma or infection.

Microscopic Features. — Out of the 20 grafts, 14 showed good and 3 fairly good viability, while 3 grafts resulted in necrosis. Because the dermal layer of this age is faster and the connective tissue cells are more fusiform, the graft could better be distinguished from the mesoderm of the membrane. Blood vessels occurred in the dermal layer abundantly, being filled with chick erythrocytes after the 2nd day. Signs referring to good viability and growth were seen especially around the hair follicles and in the epidermis where mitoses were frequent. On the surface above the peridermal layer, beginning *stratum corneum* development was observed (Fig. 7).

Five-Month-Old Human Embryonic Skin

Macroscopic Features. — The skin of this age was more like the adult skin, and it was much more difficult to place the graft on the membrane. Besides, during the inoculation its margins tended to roll penetrating into the usually hypertrophized membrane. Thus there remained a small

skin spot on the CAM where the ordinary skin folds could easily be seen. On the endodermal side of the membrane, the concave formation was pink in color and about 1 cm in diameter. On the 1st—4th days of the experiment, 2 embryos died of trauma.

Microscopic Features. — Of the 8 grafts, 5 showed good and 2 fairly good viability, while one resulted in necrosis. Because the dermal layer of the skin already was fibrillous, it was easy to distinguish the graft tissue from the membrane. In most cases, about two-thirds of the graft had sunk into the mesoderm of the membrane. Well developed epidermal and corium layers, hair follicles and even fat tissue could be noted (Fig. 8 a).

Reactions on the Membrane. — No marked differences could be observed in the reactions on the membrane between the different age groups of the grafts, and the reactions resembled those observed with the chick tissue. No foreign tissue reactions occurred, but inflammatory cells appeared only around necrotic grafts. In some preparations the well survived graft lay totally on an intact membrane.

Reactions in the Embryo. — No changes were found in the embryos in macroscopic or microscopic examinations.

Retransplantation Experiments into New Eggs

A disadvantage in the CAM method is that the tissue can be preserved on the membrane for limited periods only, as the chicken hatches on the 21st day of incubation. This becomes most clear in slowly growing tissue species. Various attempts have been made to lengthen the period the tissue is on the membrane. *E. g.*, Hurst, Cooke and McLennan (41) made efforts to cultivate tissues on duck eggs, the hatching time of which is longer than that of chick eggs, but the results did not fulfil the expectations owing to the high mortality of eggs. On the contrary, better results were obtained in experiments in which grafts were retransplanted through new egg passages (4, 48).

After having observed that embryonic human tissue could be cultivated on the membrane, the next step was to study the possibility to continue the tissue growth from one passage to another. That this would prove difficult was clear from the very beginning.

The same retransplantation method was employed in all experiments. After the maximal period of 9—10 days on the membrane the graft was cut off with a large piece of the surrounding membrane. Then the cut piece, the graft-side up, was placed in a sterile petri dish, the bottom of which was covered with a rubber plate, and which contained 20 cc of penicillin-streptomycin solution. The surface of the

rubber plate was rough, so it was easy to remove the piece from the membrane. Some of the grafts were transplanted to new eggs so that the graft and the part of the membrane surrounding it were cut in two, the other part being reserved for histologic examination. Accordingly, the behavior of the same graft tissue could be observed from passage to passage, while the other half of the graft was retransplanted to the new egg. In some cases, the graft loosened well from the membrane, but sometimes it had stuck to it so fast that the dermal surface of the graft had to be erased from the membrane with a knife. This procedure was undertaken, because it was noted in preliminary experiments that membranous remnants slowered the incorporation of the graft. After the loosening, the graft was placed on a fresh 9—11 days old membrane in the usual manner, where it stayed the maximal period of 10—11 days before a new retransplantation.

In total 20 embryonic human skin grafts were retransplanted to the second passage. Therefrom 12 grafts were implanted to the third passage, and finally 3 grafts were retransplanted from the third to the fourth passage (Table 4).

TABLE 4

Retransplantation Experiments on the Human Embryonic Skin

Number of Series	II Passage Hu $XVII_2$, Hu XX_2	III Passage Hu $XXIII_3$	IV Passage Hu $XXIV_4$	Total
Number of specimens	2	1	1	4
Number of eggs	20	12	3	35
Trauma	2	—	—	2
Infection	—	—	1	1
Good	—	—	—	—
Fairly good	3	—	—	3
Poor	15	12	2	29

Second Passage. Macroscopic Features. — It already became evident in macroscopic examinations that the second passage grafts did not show equally good viability as the grafts in the first transplantation. They were hardened, became smaller and sank easily into the membrane. Dead looking grafts occurred in abundance. They lay on the membrane like thin brown plates or a moist gray mass. Occasionally, a dead layer, probably the epidermis could be erased from the graft, while the white part of the graft remained inside the membrane. Two embryos died of trauma during this passage.

Microscopic Features. — With the exception of 3 preparations in which a few cells of the graft were still living (Fig. 9), the other 15 grafts were in necrosis. The necrotic grafts lay on the membrane wholly or in part, and the epidermis and the dermal layer could still be discerned though all cells were dead. In some cases, the grafts had sunk into the mesoderm and inflammatory cells had accumulated around them.

Third Passage. Macroscopic Features. — In total, 12 grafts were retransplanted to this passage. The size of the grafts became smaller after each passage owing to different reasons. Firstly, when retransplanting the graft into a new passage it had to be erased from the membrane and in this connection small pieces of the graft remained on it. Secondly, in some cases, the graft was cut in two for histologic examinations after the first retransplantation series. Thirdly, wrinkling and hardening occurred in the grafts. Owing to these factors, the size of the grafts in the third passage was ca. $2 \times 2 \times 0.3$ mm. Macroscopically, necrosis occurred in this passage even more often than in the second, which was probably caused by the poor condition of many grafts, though efforts had been made to select the best grafts of the previous passage. The pieces lay well on the membrane, but sank wholly or in part inside it during the 4th—5th day. The necrotic grafts did not seem to influence the mortality of eggs, as none of the eggs died during the experiment.

Microscopic Features. — All 12 grafts were dead. Some lay on and some inside the membrane. In some preparations, remnants of hairs were found, in some connective tissue still remained as a hyaloid mass, but no living cells could be found in the grafts.

Fourth Passage. — In spite of the fact that the grafts macroscopically looked dead in the third passage, the 3 best specimens were further retransplanted to the fourth passage. One of the embryos died of mold infection. The two latter grafts were entirely necrosed.

Reactions on the Membrane. — The macroscopic membranous reactions resembled those in the first series by their veil-like cloudiness and fusiform hypertrophies. In the cases in which the graft lay on the membrane like a brown dead plate the CAM was totally normal, and it was obvious that this type of dead graft could not evoke any reactions. Microscopically, around the remnants of the dying grafts inflammatory cells occurred in abundance as a sign of the reaction of the membrane.

Reactions in the Embryo. — The great mortality of the grafts did not influence the embryos, which were normal. No abnormalities were met in their organs, either. Five of the embryos were allowed to hatch, which they did quite normally.

Adult Human Skin

The adult skin grafts were obtained from 5 different sources. In two cases, the skin was taken from 2 arm stumps of 26-year-old men, amputated in connection with orthopedic repair operations. In one case, skin was obtained from a lower limb of a 60-year-old man, when the operation was necessary because of the foot gangrene caused by *thrombangitis obliterans*. In 2 cases, the grafts were obtained from normal skin covering the mammary carcinoma and from skin covering a large lipoma of women aged 45 and 50. The skin grafts were cut according to the technique reported previously and were placed on the 9—11-day-old membranes 3—4 hours after the operation. Part of the skin was transplanted to the CAM after the preservation period of several days. Results obtained through these experiments will be given later.

TABLE 5

Adult Human Skin on the CAM

Number of Series	Hu: I, V, IX, XXII, S I, II, L, LII
Number of specimens	8
Number of eggs	140
Trauma	15
Infection	1
Good	46
Fairly good	58
Poor	20

Macroscopic Features. — In total, 140 grafts were transplanted in this series. It was most difficult to expose the adult human skin on the membrane. Even if the graft lay well at first, its margins tended to roll inside the membrane. The viable graft was white, fast and entirely similar to the normal skin so that the folds were clearly seen. The dying skin was slightly bluish, moist and swollen, and the entirely dead skin graft formed a gray-green mass or a thin brown plate dried on the membrane. The macroscopic evaluation was more difficult if the graft had sunk into the membrane. The loss of eggs caused by trauma or infection was further slight being 15 in the former and one in the latter case. Altogether, two cases of infections occurred, but only one of the embryos was dead. The causative organism was *Micrococcus albus*. In the other egg, the membrane was normal and the embryo was alive, but the dead graft was totally covered with a mold.

Microscopic Features. — In total, 124 grafts were left for microscopic examination. Of them, 46 showed good viability comparable to the normal skin. In 58 cases, the greatest part of the graft survived. Only 20 grafts resulted in necrosis. In viable grafts (Fig. 10), the cells were similar to the normal, and both in the epidermis and in the connective tissues of the corium mitoses occurred referring to the growth of the graft on the membrane. The maximal period of time the graft was on the membrane was 9—10 days, and during this period degenerative changes only occurred in the group with fairly good viability. The epidermis became thinner, the fibrils occasionally swelled, and cells in the connective tissue were scarcer than before. In entirely dead grafts, the epidermis was represented by a homogeneous corneal layer in which the contours of the cells could still be detected, while the connective tissue of the corium had degenerated to a hyaline mass. Various factors evidently caused the death of the grafts, *e. g.*, the rolling of the margins since the rigid epidermis mechanically prevented the penetration of the membranous vessels into the graft. Hematoma formations also retarded the fastening of the graft on the membrane. Moreover, in agreement with previous workers (59) it was also noted that the thicker grafts were less viable than the thinner. Obviously there were also other factors, such as, above all, strange environment, because some preparations with ideal growing possibilities still showed poor viability.

Reactions on the Membrane. — As mentioned previously, the graft remained on the membrane in most cases. The macroscopic reactions of the CAM did not at all differ from the previous ones. The reactions were the following: at first a veil-like cloudiness that in the course of time turned to a fusiform grayish membranous hypertrophy around the graft. Occasionally, a narrow ring grew from the membrane over the margins of the graft, and the membranous blood vessels were as if drawn to the graft. In some cases the graft remained on the entirely intact membrane. Microscopically, the ectoderm of the membrane was swollen in the usual manner, and began its degeneration on the 3rd day being disappeared on the 4th—5th days. In the corium of the graft, the connective tissue fibers interlaced with the membranous mesoderm, and the blood vessels penetrated into the graft. If the grafts grew well, no foreign body or inflammatory reactions were found on the membrane or in the graft. If the grafts, on the contrary, were less viable usual inflammatory cell accumulations occurred in the membrane, and giant cells appeared around the dead tissue remnants. They were met with in earlier experiments, too, so it was no specific phenomenon, but now they were much more numerous. Likewise, epithelial pearls

were met frequently, but in this case it seemed likely that they developed through the sinking of the epithelial islands of the graft. Papillary hypertrophies were met in the ectoderm and endoderm of the membrane, mostly occurring on the membranes of 13 days of age or older. In the ectoderm the proliferation took place close to the margins of the graft, in the folds of the membrane, and in the endoderm especially opposite to the degenerated grafts. In the mesoderm, the usual mesenchymal proliferation was noticed. Around the less viable grafts, the membrane sometimes formed a demarcation line, which never occurred round the grafts that were in a good condition.

Reactions in the Embryo. — In one embryo the bones of the skull were poorly developed so that the brains were only covered with a thin transparent connective membrane. In addition, the right lobe of the liver was about half of the normal size. Similar anomalies, however, have even been observed among normal embryos (74) and obviously these phenomena also belong to them.

Retransplantation Experiments into New Eggs

Goodpasture and co-workers (26) reported on successful transplantations of skin grafts to the second passage. In their experiments, the skin survived on the membrane for 14 days altogether. Blank, Coriell and Scott (4) later succeeded in transplanting skin through four passages by using extremely thin grafts containing epidermal tissue only. Thus they cultivated the skin on the membrane for 27 days in total. In this study, attempts have also been made to maintain adult human skin tissue on the CAM as long as possible using the retransplantation method (Table 6).

TABLE 6

Retransplantation Experiments on the Adult Human Skin

Number of Series	II Passage HuI_2, V_2, IX_2, $XXII_2$	III Passage HuV_3, IX_3 $XXII_3$	IV Passage $HuXXII_4$	V Passage $HuXXII_5$	Total
Number of specimens	4	3	2	—	9
Number of eggs	42	11	6	5	64
Trauma	—	1	—	—	1
Infection	—	—	—	—	—
Good	—	—	—	—	—
Fairly good	13	4	—	—	17
Poor	29	6	6	5	46

Second Passage. Macroscopic Features. — In total, 42 grafts from the original series were implanted to the second passage and they were let to lie on the membrane for 10 days. The majority of the grafts sank into the membrane making the macroscopic evaluation difficult. When the graft still lay on the membrane, the dead graft was macroscopically easy to recognize as a yellow-brown plate or a sticky greenish mass. Contrary to the observations made by Goodpasture and co-workers (26), no infections could be shown in dead grafts which would have explained the death. Neither did there occur any deaths in the eggs caused by infections or trauma, so that all 42 grafts could also be examined microscopically.

Microscopic Features. — Entirely normal skin tissue was not found, 13 grafts showed fairly good viability and the remaining 29 grafts resulted in necrosis. Signs of degeneration were seen both in the epidermis and corium. The epidermis became considerably thinner containing only few cellular layers. Its nuclei swelled and vacuoles occurred in abundance in the cytoplasm. The fibrils in the corium became swollen and less marked, and a great many inflammatory cells were found between them. Probably this also referred to the fact that the grafts showed poor viability on the membrane (Fig. 11).

Third Passage. Macroscopic Features. — Altogether 11 grafts were implanted from the second passage to the third. The second passage gave better results in the retransplantations of the adult skin than of the embryonic tissue. This proved to be the case when studying the grafts of the third passage, too. The macroscopic findings were divided into 2 groups. The graft usually sank into the membrane forming a gray or pink fusiform formation, and it was difficult to evaluate the viability of the graft. It is worth emphasizing that the skin tissue, which during the first series remained on the membrane almost without exceptions, tended to sink into it in the second and especially the third passages. Only a small group of grafts lay on the membrane usually as a sticky gray-green mass or as a dried black plate indicating that the graft was necrosed. Not a single piece of skin in so good a condition as in the first passage was met with. In this series, no infections occurred and only one embryo died of trauma.

Microscopic Features. — None of the 10 grafts were comparable to the normal skin. In 4 grafts, some of the epithelial cells were viable, while 6 grafts were entirely necrosed. As compared with the second passage, the viability of the grafts was still poorer. In some preparations, the connective tissue had formed a red ball inside the membrane.

Fourth Passage. Macroscopic Features. — In total, the 6 best looking

grafts were retransplanted to this passage. During the passages the graft was hardened to the extent that it was difficult to handle. The piece exposed well on the membrane, but when examined with the magnifying glass through the Scotch tape window it was noticed that it slowly adhered to the membrane on the 4th—5th day and looked dead. The majority of the grafts later sank into the membrane making the macroscopic evaluation difficult.

Microscopic Features. — All the 6 grafts were necrosed, and the connective tissue, the structure of which still could be detected, had formed a homogeneous ball inside the mesoderm of the membrane. Not a sign of the epidermis of the skin could be seen.

Fifth Passage. — For control purposes, 5 grafts were further implanted from the fourth passage to the fifth. Two embryos died of trauma, and the remaining 3 grafts were macroscopically found to be necrosed. This fact was also confirmed microscopically.

Reactions on the Membrane. — From the second passage on, the hardened grafts sank easily into the membrane, and the membranous ectoderm tended to cover them. In the microscope, the membranous reactions resembled those of fairly good and necrosed grafts met in the first series. Both demarcation line, inflammatory and giant cells occurred around almost all grafts. In the third passage, and especially in the fourth and fifth passages, the grafts mostly lay as brown plates on the membrane.

Reactions in the Embryo. — All the embryos were normal. No changes were met with in histologic specimens, either.

Influence of the Preservation Time of the Human Skin upon the Viability of the Graft on the CAM

Murphy (57) and later Bender, Friedgood and Lee (2) assumed that the necrosis of the human tissue on the membrane was due to the elapse of time before the graft could be implanted on the membrane. Goodpasture and co-workers (26) reported on the basis of only a single experiment, it is true, that the human skin gave excellent takes after the preservation at 5° C for 24 hours. Moreover, Blank, Coriell and Scott (4) preserved the skin at 4° C for even 5 days before the implantation. Matthews (54) preserved the skin in an airtight place at about 3°—6° C for seven months observing no major changes in it. He also used human skin autografts successfully for the treatment of burns after 21 days' preservation period, and reported that the skin, in spite of the preservation period of 8 days, maintained its viability in hanging drop culture.

In 1954, Brown and Fryer (5) reported that *post mortem* skin has been found to be viable up to one month by folding it on itself and keeping it moist in a sterile jar at ordinary icebox temperature above freezing. Accordingly, it was considered to be of interest to examine if and to what extent the time factor influenced the viability of the graft on the membrane (Table 7).

TABLE 7

Refrigerated Adult Human Skin on the CAM

Number of Series	Hu X	Hu II	Hu VII	Hu III	Hu IV	Hu VIII	Total
Number of specimens	1	1	1	1	1	1	6
Preservation time of the skin (days) ..	1	3	5	7	11	15	1—15
Number of eggs ..	11	12	14	14	25	11	87
Trauma	1	—	1	—	4	1	7
Infection	—	—	—	—	—	—	—
Good	5	3	6	4	—	5	23
Fairly good	1	6	7	8	6	3	31
Poor	4	3	—	2	15	2	26

Material and Methods. — The same skin tissues as were previously used in adult fresh-skin experiments were employed as material. The amputated extremity was preserved in the refrigerator at 5° C. For the sake of certainty, skin was taken from 3 extremities, *i. e.*, from two upper and one lower. The former amputations were performed in connection with repair operations of defective arm stumps, and the amputation of the lower extremity was made because of the foot gangrene caused by *thrombangitis obliterans*. The skin was cut tangentially using the same method as in fresh-skin experiments. The skin grafts from the refrigerated extremity could be cut ca. 0.3—0.5 mm thick, and after soaking in the penicillin-streptomycin solution they were placed on the 9—11 days old membrane in the usual manner.

Macroscopic Features. — The grafts, being somewhat moister, more grayish and swollen than the fresh-skin grafts, remained on the membrane as a rule. In spite of these changes, it was obvious that the grafts lived on the membrane. Differences occurred between various series, but they did not influence the final results. The grafts cut from the lower extremity lived best (series Hu VII and Hu VIII), while the grafts from the arm stumps (series Hu X, Hu II, Hu III) were less successful. The

refrigerated skin tissue did not increase the mortality of eggs. It is worth emphasizing that no infections occurred (Table 7, p. 47).

Microscopic Features. — The microscopic picture was exceptionally good, even better than could have been expected. The most important observation was that the influence of the time factor was smaller than was presumed previously (26, 4). *Viz.*, the refrigerated grafts, preserved for 15 days, were similar to those implanted on the membrane after a shorter preservation time. *E. g.*, in the 15-day series, the number of successful grafts was almost higher than in the series where grafts were preserved for one day only. Corresponding to macroscopic findings, the behavior of grafts varied slightly according to the extremity they were taken from. The grafts taken from the lower extremity were most successful (Fig. 12 a and b), while more degenerative changes were found in grafts taken from the arm. The number of successful grafts was probably slightly decreasing as compared with the fresh-skin tissue, and in the cells of the grafts slight decrease of cell protoplasm was observed. However, mitoses were also met with in these grafts as signs of growth.

Reactions on the Membrane and in the Embryo. — Inflammatory cells occurred abundantly on the membrane around the viable grafts, too, so that in this respect the reactions differed from those of fresh-skin grafts, in which nothing abnormal was found around the living grafts. Nothing remarkable was noted in the embryos. No embryos were infected.

Summary

The embryonic human skin tissue showed fairly good viability on the membrane. No marked differences were found in the behavior of the human embryonic skin tissue grafts of various ages. As compared with the control tissue, the greatest changes were observed on the dermal layer, which was thickened because of the cell multiplication and edema.

The adult human skin grafts differed from the embryonic skin tissues in their smaller mitotic activity, though this phenomenon might be due to the basic structure of the tissue. The adult human skin also showed relatively good viability on the membrane. Of the transplanted grafts ca. 82 per cent survived, the figure corresponding to the results obtained with the embryonic skin tissue. Equal to embryonic skin tissue grafts, the adult grafts mostly remained on the membrane whereby the well surviving grafts were macroscopically entirely similar to the normal skin. This was also the case when examined microscopically.

No specific foreign body reactions were found in the chicken egg to the heterologous tissue and the changes occurring in the membrane were similar both in embryonic and adult tissue transplantations.

In the retransplantation experiments, it proved successful to maintain the embryonic tissue on the CAM through two passages, *i. e.*, for 20 days, while the experiments performed on the adult tissue revealed better results. *Viz.*, it could be transplanted through three passages, in other words, it survived on the membrane for 30 days.

Adult human skin, preserved at 5° C for 15 days, was also transplanted on the membrane. It was found that even then the tissue maintained its capacity for living and was good for grafting. The grafts taken from the lower extremity were most successful. Because here the cause for the amputation was *thrombangitis obliterans*, it could be presumed that the grafts showed better viability because the skin of the lower extremity had become used to poor circumstances and thus survived better the preservation time and transplantation. As compared with the fresh-skin grafts, the results were but slightly poorer, and better than could have been expected.

When the results were compared with those of the homologous tissue, it was found that the skin of chick embryo and chicken lived on the membrane slightly better than the human skin, but the viability of the skin of adult hen and of human skin was almost the same.

Benign Tumors of the Human Skin

The tumors of this group were selected from the generally occurring tumors in the surgical out-patients' department. Among them there were entirely benign tumors, such as fibromas and naevi, but also more precancerous tumors (leukoplakias) (Table 8).

TABLE 8

Benign Human Skin Tumors on the CAM

Tissue	Keloid	Fibroma	Naevus	Verruca	Leukoplakia	Total
Number of tumors	1	3	7	4	5	20
Number of eggs ..	18	38	40	47	15	158
Trauma	4	6	5	12	2	29
Infection	—	2	—	—	1	3
Good	6	8	8	—	—	22
Fairly good	7	12	16	21	—	56
Poor	1	10	11	14	12	48

Keloid Tissue

Goodpasture and co-workers (26) also cultivated keloid and naevus tissues on the membrane. They reported that these showed almost better viability than the normal skin, which was probably due to the suitability of the structure of keloid and naevus tissues for incorporation. In the present study, experiments were performed on the keloid tissue of a 10-year-old boy. The grafts of the keloid scar, developed after burns, were of the same size as those taken from the skin and were transplanted to the 9—11-days-old membrane. The results mostly agreed with those obtained by Goodpasture and co-workers (26).

Macroscopic Features. — In total, 18 grafts were transplanted, most of which sank wholly or in part into the membrane on the 3rd day. In this respect, the grafts differed from those taken from the skin. As the majority of the grafts was inside the membranous proliferation, the macroscopic evaluation was difficult, but the grafts seemed to have good viability. They appeared transparent and white through a fusiform formation. Four of the embryos died of trauma, but no infections occurred.

Microscopic Features. — The keloid tissue showed equally good viability on the CAM as the normal skin tissue. Of the 14 grafts, 6 showed good and 7 fairly good viability, while one graft resulted in necrosis. In the blood vessels of the successful grafts, the blood cells of the chick embryo were found from the second day on and on the 5th day the capillary bed of the membrane began to interlace with the connective tissue fibrils. When compared with the control preparations, the connective tissue of the successful grafts showed a tendency to undergo lysis and the fibrils seemed narrower, perhaps indicating that the conditions, where the graft was living, were not satisfactory.

Fibroma Tissue

The tissue was taken from 3 tumors of fibroma type.

Macroscopic Features. — The structure of the fibroma tissue does not greatly differ from the keloid and, accordingly, the results were mostly equal to those obtained in the previous series. In total, 38 grafts were transplanted and the majority of them remained on the membrane for the whole test period. During the experiment, 6 embryos died of trauma and 2 of infection, the causative organism of the latter being a *Micrococcus*.

Microscopic Features. — Thirty embryos survived till the end of the experiment. On the membranes, 8 grafts showed good and 12 fairly good viability, while 10 grafts resulted in necrosis. The results were thus slightly poorer than those obtained with the normal skin.

Naevus Tissue

This tissue was transplanted from 7 different naevi which were transported like all other specimens to the laboratory in physiological saline in order to avoid drying. There they were transplanted on the membrane within 2—3 hours after the operation.

Macroscopic Features. — The grafts could be seen on the membrane as dark-brown pieces. On the 2nd—3rd day some of the grafts sank into the membrane and developed there a usual fusiform formation. In total, 40 grafts were transplanted, and 5 embryos died of trauma. No infections occurred.

Microscopic Features. — The remaining 35 grafts were examined microscopically. Eight of them showed good and 16 fairly good viability, while 11 resulted in necrosis. The results were thus slightly poorer than those obtained with the skin tissue, perhaps because these grafts could not be cut so thin as the skin grafts. The epidermal and corium cells were similar to those in the ordinary skin grafts. Normal pigmentary cells could also be seen, though they were mostly spread, because pigment was noticed to be free in the tissues and macrophages (Fig. 13 a and b).

Verruca Plantae Pedis Tissue

In four cases of verruca of the sole, the tumor was removed by an oval excision. The grafts were then cut tangentially as thin as possible, ca. 1 mm. The bulk of the verruca tissue was partially dead epidermis and thus not suited for transplantation. As verrucae are supposed to be caused by a virus, it was interesting to note whether the sensitive membrane was able to respond specifically.

Macroscopic Features. — In total, 47 grafts were transplanted on the membrane. Twelve embryos died of trauma so that 35 grafts remained for the evaluation of transplantation results. High mortality in this series was probably due to the rigid grafts causing more damage on the CAM than skin grafts. However, the existence of some damaging agent cannot be left without consideration. No inclusion bodies or other abnormalities could be found on the membrane or in the embryo. Usually, the grafts remained loose on the membrane rather long, even for 4 days, as a sign of the slow incorporation. The majority of the grafts later sank into the membrane forming an almost sac-like formation.

Microscopic Features. — In 5 of the 35 grafts, the cells of the hypertrophized epidermal layer were relatively viable and in 16 grafts cells were surviving both in the epidermis and especially in the connective

tissue underneath. However, all of these grafts were considered only fairly good because part of the graft was in necrosis (Fig. 14). The remaining 14 grafts were entirely necrosed. No specific changes occurred in the embryos or membranes.

Leukoplakia Tissue

Of the 5 leukoplakia specimens, 3 were taken from the mucous membrane of the cheek and 3 from the lower lip. The examination of the original tissue revealed such a great cellular polymorphism in basal parts of the epithelium of two tissue grafts that the tissue was considered precancerous. In one tissue graft, the inflammatory cell infiltration proved to be strong, and in four others only moderate.

Macroscopic Features. — In total, 15 grafts were transplanted to the membrane, but because 2 embryos died of trauma and one became contaminated with *Micrococcus albus*, 12 grafts remained. In macroscopic examination, the majority was found to be in necrosis, as the grafts lay on the membrane as a dead yellow mass.

Microscopic Features. — All the 12 grafts resulted in necrosis lying as a homogeneous mass both on and inside the membrane. The membrane showed strong inflammatory cell reaction and often a strikingly noticeable demarcation line around the dead grafts.

Reactions on the Membrane. — The reactions did not differ from the earlier ones, being similar in all these tissue groups. Most of the inflammatory cells occurred in leukoplakia tissue grafts. Since the mortality of the grafts was highest in this group it is obvious that the reaction was caused by the dead grafts.

Reactions in the Embryo. — As compared with the previous experiments the mortality of the embryos was also relatively high (Table 8, p. 49). No changes in the embryos or in their organs were found in macroscopic and microscopic examinations. In the 5 cases, in which the embryos were allowed to hatch, the chickens grew normally.

Summary

When comparing the grafts of benign tumors with the skin grafts the viability of the former was slightly poorer. This could be due to the difficulty to cut these grafts as thin as the skin grafts. The tumors most resembling the normal skin showed the best viability on the

membrane, while the other tissues (verruca and leukoplakia grafts) were less viable. However, the possibility that the structure of the graft tissue may play a certain role in the poor viability has to be taken into consideration. The latter two tissues contained dense epidermis inhibiting the penetration of the membranous blood vessels.

Malignant Tumors of the Human Skin

In these experiments, the basocellular carcinoma tissue was mostly used and it was taken from 30 different tumors. The tissue from 15 different spinocellular tumors was also transplanted. In addition to these major groups, 3 baso-spinocellular and 3 anaplastic skin carcinomas and for comparison grafts from 3 mammary carcinomas adherent to the skin were transplanted. Finally skin carcinoma tissue and normal skin tissue were transplanted side by side on the same membrane. The majority of the tumors was taken by means of conchotome. Because specimens were also to be reserved for histological diagnosis, the piece left for transplantation was rather small, about $6 \times 7 \times 3$ mm in size. To avoid drying, the specimens were transported to the laboratory in physiological saline. The transplantation was performed 3—4 hours after the operation the grafts being soaked prophylactically in penicillin-streptomycin solution. In this solution they were cut into pieces ca. $3 \times 3 \times 0.5$ mm in size, and then transplanted to the 9—11 days old membranes (Table 9).

TABLE 9

Malignant Human Skin Tumors on the CAM

Tissue	Ca. Baso-cell.	Ca. Spinocell.	Ca. Baso-spinocell.	Ca. Anapl.	Ca. Mammae	Total
Number of tumors .	30	15	3	3	3	54
Number of eggs ..	130	80	8	22	41	281
Trauma	9	9	—	3	1	22
Infection	10	3	1	—	—	14
Good		1				1
Fairly good	3	4	1	—	2	10
Questionable	21		—	2	5	28
Poor	87	63	6	17	33	206

Basocellular Carcinoma Tissue

Macroscopic Features. — It was even seen with the naked eye that the basocellular carcinoma tissue did not survive on the membrane so well as the normal skin. Several grafts resulted in necrosis. The necrotic grafts lay on the membrane as a dry yellow plate or a gray-green mass and around the necrotic formation a small annular vesicle 6 mm in diameter, containing yellowish fluid, was occasionally met with. The vesicle formation was a new type of reaction of the membranes. The evaluation of the living grafts was difficult as they had often sunk into the membrane causing there the usual fusiform formation. The mortality of embryos was higher than in normal tissue transplantations, and above all, the number of embryos died of infections was great as compared with the previous series. Of the 130 eggs used 9 died of trauma and 10 of infections. The most general causative organisms were *Micrococcus saprophyticus var. albus* (5 cases), and thereafter *Micrococcus aureus* (1 case), a diphtheroid (1 case), *sarcina* (1 case), and a short gram-negative rod (1 case). A mold occurred sometimes in the dead grafts, causing the death of the embryo in only one case for certain. The tumor specimens were infected more frequently than the normal skin, and the penicillin-streptomycin solution was obviously not effective enough to prevent the infection in all cases.

Microscopic Features. — The microscopic examination confirmed that the malignant tissue did not survive on the membrane so well as the normal skin tissue. Until the end of the series lived 111 embryos, and on the membranes only 3 fairly viable grafts were met with. It was possible that some malignant cells still survived in 21 grafts, while in 87 cases the entire graft or its malignant part was totally necrosed. Necrosis was thus met with in all grafts and it seemed to be increasing from the first day on, while in normal skin grafts necrosis occurred noticeably slighter. The viable malignant tissue lay in the membranous mesoderm, not upon it, as the normal skin graft (Fig. 15 a and b). In the grafts in which some malignant cells possibly survived they were always found inside the mesoderm along the margins of the necrotic area. The normal connective tissue of the graft survived better than the malignant tissue itself, which was often split into a net-like cell structure contained poorly staining tissue where the structure of malignant cells was hardly detectable. Here the living cells were mainly inflammatory cells of the membrane reflecting perhaps a positive taxis due to the malignant, dying or dead cells (Fig. 16 a and b).

Retransplantation Experiments into New Eggs

Second Passage. — In the hope that possible adaptation to the membrane might occur in basocellular carcinoma tissue and that human tumor tissue surviving in the egg might thus be obtainable, the 7 best looking grafts were implanted from the first series to new eggs. The grafts were removed from the membrane and cleaned from the membranous remnants so that they were ca. $2 \times 2 \times 0.5$ mm in size. Unfortunately, they were much harder than when grafted on the CAM for the first time (Table 10).

TABLE 10

Retransplantation Experiments with Basocellular Carcinoma Tissue

Passage	FC II_2	FL XXI_2
Number of tumors		2
Number of eggs		7
Trauma		—
Infection		—
Fairly good		—
Questionable		—
Poor		7

Macroscopic Features.— The regrafted specimens looked dead without exceptions. A dried brown plate lay on the membrane, which hardly reacted to it. All the embryos were living.

Microscopic Features. — Corresponding to the macroscopic examination, all the 7 grafts were dead within 10 days. A mesodermal bulge had occasionally grown in them. Other retransplantation efforts were not considered justified.

Spinocellular Carcinoma Tissue

Macroscopic Features. — In total, 80 grafts of 15 different spinocellular carcinomas were transplanted to the CAM. The macroscopic picture did not greatly differ from that of the basocellular tissue. Only in 2—3 cases, viable looking tissues were met with on the membrane. If the graft was not on the membrane, it had sunk into it causing a fusiform formation and making the macroscopic evaluation difficult. Mortality by infection and trauma was higher than when transplanting the normal tissue. Of the 80 eggs used, 9 embryos died of trauma and 3 of

infections. The causative organism was in 2 cases *Micrococcus saprophyticus var. albus* and in one a mold. Moreover, mold occurred in 5 dead grafts, though it had had no clearly unfavorable influence on the embryo.

Microscopic Features. — In total, 12 embryos died of infections and trauma, so that 68 remained for microscopic examination. In the membrane, living malignant tissue occurred in 1 graft and in 4 grafts a few malignant cells survived, while 63 grafts were totally necrosed. The first mentioned graft, being in a good condition, had been on the membrane for 8 days. It was partly necrosed, but in an epithelial ball the cell division was active. The cells were atypical so the graft had to be considered malignant tissue (Fig. 17 a). The preserved cells of the 4 grafts belonging to the questionably viable group were usually between the graft and the membrane or they sank into the membrane, while most of the graft lay on it as a necrotic lump. The dead grafts usually appeared on the membrane as a homogeneous yellow mass or had partly sunk into the mesoderm, where the membrane formed a demarcation line around the dead tissue. As a rule, it seemed that the membrane tended to remove the dead graft as soon as possible, for the membranous reaction could easily be seen very often in transplantation experiments of the malignant tissue.

Retransplantation Experiments into New Eggs

Second Passage. — As in the basocellular tissue group, in this series 7 grafts were retransplanted to new membranes from 3 different spinocellular carcinoma grafts (Table 11).

TABLE 11

Retransplantation Experiments with Spinocellular Carcinoma Tissue

Series	II Passage FL $XXIV_2FXCII_2FLXXXI_2$	III Passage FL $XXXI_3$	Total
Number of tumors	3	1	4
Number of eggs ..	7	5	12
Trauma	—	—	—
Infection	—	—	—
Fairly good	—	—	—
Questionable	—	—	—
Poor	7	5	12

Macroscopic Features. — Macroscopically, it did not seem to be very promising to retransplant malignant tissue to the new egg passages. The majority of the grafts lay on the membrane as a necrotic mass and in a few cases possibly viable tissues occurred inside the membranes.

Microscopic Features. — Microscopically, the results proved to be similar to those obtained in the basocellular carcinoma series. Six grafts were totally necrosed on the membrane, and in one graft part of the tissue had sunk into the mesoderm forming a hyaline mass, which was surrounded by the inflammatory cell accumulation of the membrane.

Third Passage. — For purposes of control, 5 grafts were retransplanted from the second passage to the third, but all the grafts proved to be dead both in macroscopic and microscopic examinations.

Baso-spinocellular Carcinoma Tissue

Macroscopic Features. — In total, 8 grafts of baso-spinocellular carcinoma tissue taken from 3 different tumors were transplanted to the CAM. One of the embryos died of infection by *Bacterium paracoli*, and 7 grafts remained for the microscopic evaluation. It was confirmed macroscopically that 6 grafts were necrosed on the membrane, while in one graft at least part of the tissue showed good viability.

Microscopic Features. — According to macroscopic evaluation, total necrosis occurred in 6 grafts, while in one graft, which survived on the CAM for 10 days, part of malignant tissue was still viable. The cells were mainly of spinocellular carcinoma type.

Anaplastic Carcinoma Tissue

Macroscopic Features. — In total, 22 grafts were transplanted to the membrane from 3 different tumors. Macroscopically the grafts differed from the previous carcinoma types in their jelly-like appearance. They easily adhered to the membrane and mostly lay upon it. Most of them were transparently white and it seemed that they did not survive on the membrane very well. Three embryos died of trauma, and no infections occurred.

Microscopic Features. — Nineteen chick embryos lived until the end of the series and 2 grafts belonged to the doubtfully viable group. In the remaining 17 grafts no malignant cells survived. The anaplastic carcinoma tissue showed a tendency to undergo lysis rapidly inside the mesodermal proliferation of the membrane (ca. within 3 days), and the connective tissue of the graft also seemed to be destroyed sooner than

usual. This was perhaps due to the fact that the connective tissue was evenly infiltrated by the carcinoma tissue and died together with it. The membranous blood vessels penetrated abundantly into the destroyed grafts. Because of the small amount of the tissue no retransplantation experiments were performed.

Mammary Carcinoma Tissue

Macroscopic Features. — Forty-one fragments were grafted from 3 *carcinoma solidum* types adherent to the skin of the breast. As compared with the former, the macroscopic finding was more promising. The microscopic examination showed that this was, however, due to the rich normal looking connective tissue of the graft, which was extremely viable on the CAM. All the grafts had, at least in part, sunk into the membrane. No ulcers existed in the skin infiltrated by the tumors. This probably also influenced the sterility of the specimens as none of the eggs were infected. Only one embryo died during the experiment, accordingly, the number of traumatic deaths was insignificant.

Microscopic Features. — Forty grafts were examined microscopically. In 2 of them the malignant part of the tissue was fairly viable, and in 5 some probably viable malignant cells still survived, while in the remaining 33 at least the malignant and sometimes even the normal tissues were dead. All the grafts were surrounded by the membranous mesoderm. Surviving tissue was not found on the membrane. In this series, there was a chance that malignant tissue was not included in grafts as the malignant cells did not infiltrate the tissue specimen as uniformly as in skin tumors. This has to be kept in mind in the evaluation of the results. On the other hand, the original place of malignant cells was often found in the middle of the connective tissue of the graft, but during the stay on the membrane the malignant tissue had died and become homogeneous. The normal connective tissue of the mammary gland thus survived better than the malignant cells, though hyalinization occurred in it, too. The surviving connective tissue fibrils again interlaced with the fibrils of the mesoderm and 3—4 days after the inoculation capillaries of blood vessels penetrated into the graft. The fat tissue of the graft also seemed to survive well. The inflammatory cell reaction of the membrane was smaller than in the previous carcinoma experiments.

Retransplantation Experiments into New Eggs

Second Passage. — In total, 9 grafts were retransplanted from the first series to the second passage (Table 12).

TABLE 12

Retransplantation Experiments with Mammary Carcinoma Tissue

Number of tumors	3
Number of eggs	9
Trauma	1
Infection	—
Fairly good	—
Questionable	—
Poor	8

Macroscopic Features. — The grafts were grayer in color and harder than the original, but lay, however, well on the membrane and sank into it on the 3rd—4th day, which made the macroscopic evaluation difficult. One of the embryos died of trauma during the experiment, but no infections occurred.

Microscopic Features. — In the remaining 8 grafts the connective tissue survived fairly well, though hyalinization occurred here and there. From the 2nd day on, the mesodermal cells and blood capillaries of the membrane penetrated, as usual, into the connective tissue of the graft. Malignant cells were not found in a single graft. Therefore further passage efforts were not considered justified.

Reactions on the Membrane. — Since the reactions of all malignant tumors were similar, they can be discussed together. Macroscopically, the living grafts at least had a greater tendency to sink into the membrane than the corresponding human and chicken skin grafts. The usual white, veil-like membranous reaction which later turned to a hard fusiform hypertrophy was mostly stronger than that in the skin transplantations. Microscopically, the cell reaction proved to be stronger in all tumor experiments than in those performed with human and chicken skins. Inflammatory cells were seen in the graft itself, especially in its margins and in the surrounding mesoderm. They were most abundant in the membranes of the dead grafts. In the membranes of the more viable grafts the cell reaction was smaller but occurred even there more frequently than in normal skin experiments. In the further passage, when sometimes only a dead yellow homogeneous lump was

met with, the membranous mesoderm was sometimes entirely normal indicating that the dead piece in itself was not capable of stimulating the membranous reaction. In addition to rich inflammatory cell accumulations, plenty of giant cells occurred around the dead grafts and they formed, together with dying graft remnants and a rich capillary network, a formation macroscopically resembling a small abscess. Moreover, around some grafts the membrane formed a demarcation line as if it had attempted to free itself from the graft. It is difficult to say whether this reaction was provoked by the viable malignant graft and thus caused its destruction, or whether it was a reaction to the dead graft only. The latter assumption seems, however, more likely.

Reactions in the Embryo. — The mortality of embryos was higher than in the previous series. Above all, the number of embryos that died of infections increased considerably. Too many or too resistant bacteria might have occurred in cancer tissue. The number of deaths caused by trauma, ca. 8 per cent, was mainly corresponding to the results obtained with normal skin tissue experiments. The malignant tissue grafts thus showed no increasing influence on the mortality of embryos. Neither did the macroscopic and microscopic examinations indicate that the malignant tissue would have caused changes in embryos, as all findings both in the living and dead embryos were normal. No metastases were found in specimens taken from the liver, spleen or lungs. The 5 chickens that were let to hatch in each series lived well.

Malignant and Normal Human Skin Tissue on the Same CAM

As it was of interest to compare the behavior of tumor and skin grafts on the same membrane and examine their possible mutual influences, a series was conducted in which both grafts were transplanted simultaneously to the same membrane. The carcinoma tissue was taken from 3 basocellular and 3 spinocellular carcinomas and treated like previous cancer tissues. The skin grafts were obtained in connection with surgical operations and they were transported to the laboratory in physiological saline like the cancer specimens. The transplantation to the 9—11 days old eggs took place 2—3 hours after the removal of the tissue (Table 13).

TABLE 13

Malignant and Normal Human Skin Tissue on the Same CAM

Tissue	Ca. Basoc.	Skin	Ca. Spinoc.	Skin
Number of specimens	3	3	3	3
Number of eggs ..	11	11	14	14
Trauma	1	1	2	2
Infection	1	1	1	1
Fairly good	—	—	—	1
Questionable	—	7	—	4
Poor	9	2	11	6

Basocellular Carcinoma and Normal Skin

Macroscopic Features. — The greater part of both grafts remained on the membrane. The skin graft seemed to be more viable than the cancer graft, which on the 10th day had decreased in size nearly one third as compared with the skin graft (Fig. 18). The cancer graft looked necrotic and was browner than the skin graft. Occasionally, it seemed that the skin graft was also less viable on the membrane with the malignant tissue as compared with our previous experience on skin grafts.

Microscopic Features. — The normal skin tissue did not increase the viability of the malignant tissue on the membrane. As a rule, the malignant tissue turned to a poorly staining yellowish mass or totally underwent lysis inside the membrane. In only few cases, the living connective tissue still survived. The microscopic features verified the macroscopic observation that the skin grafts generally survived better on the membrane than the malignant tissue. However, necrosed skin grafts were frequently seen indicating that the skin tissue showed poorer viability on the membrane when connected with the malignant tissue than alone (Fig. 19). No clear fusion between the grafts could be seen.

Macroscopic Features. — Macroscopically, the grafts in this series did not differ from the previous ones in any way. The carcinoma graft was yellowish and smaller than the skin graft, which was white and showed fair viability. When the grafts sank into the membrane they could be seen as hard gray pieces through the fusiform hypertrophy formed by the membrane. Two embryos died during the experiment, and in one of them a mold was found. Possibly the mold was not the actual cause of the death as the embryo may have died earlier.

Microscopic Features. — Altogether 12 grafts were left for the microscopic examination. Malignant tissue proved to be dead or to have disappeared entirely, while about half of the skin grafts were alive and half were necrosed. The connective tissue of the skin survived better on the membrane, and normal connective tissue or a ball formed by more or less swollen connective tissue fibrils could be seen in the preparation. However, the connective tissues of the skin and the epidermis were sometimes equally degenerated. No clear fusion was met with between the grafts in this series, either.

Reactions in the Egg and Embryo. — The membranous reaction did not differ from that in the previous series. Nor did the mortality of embryos increase in spite of the fact that 2 grafts were transplanted instead of one.

Summary

The malignant skin tumor grafts showed considerably poorer viability on the membrane than the normal skin and benign skin tumors. No clear difference was found between the different malignant tissue groups. Perhaps the spinocellular carcinoma tissue had the best viability. As a rule, the normal tissue component of the malignant graft was more viable than the malignant part, the phenomenon best to be seen in mammary carcinoma tissue implantations. When the connective tissue was infiltrated by malignant cells, it degenerated with the dying malignant tissue. The malignant tissue grafts tended to sink into the membranous mesoderm more easily than the skin grafts. The membrane reacted to the dying tissue remnants with abundant inflammatory and giant cell formations and a demarcation line. The grafts that died in the incorporation phase remained on the membrane as dried plates. The attempts to retransplant malignant tissue to new eggs and adapt the tissue

were not successful. No changes indicating metastases of the malignant tissue or other unfavorable reactions were met with in the embryos. On the other hand, many embryos died of infections which were probably caused by higher contamination frequency in the malignant tissue. In the experiments where the malignant and normal skin tissue lay side by side on the same CAM the graft containing malignant tissue showed again poorer viability on the membrane than the normal skin tissue, which was not able to promote the viability of the malignant tissue on the membrane. The results were, *viz.*, almost the same as if solely malignant skin grafts had been transplanted on the membrane. On the other hand, the normal skin tissue seemed to show poorer viability in these series with the malignant tissue. No fusion was found between the grafts. The changes occurring in the membrane and embryos did not differ from the previous experiments.

DISCUSSION

As mentioned in the introduction to this work, the results obtained from grafting heterologous tissue to the CAM of the embryonated chicken egg have been slightly diverging (57, 94, 41, 47, 10, 48, 49). For this reason, the present study was performed with the main purpose to examine closer the different factors affecting the viability of the homologous and heterologous skin tissue on the CAM. This was done partly with the hope to establish continuous growth of human tumors in the embryonated egg and partly with the intention to compare the viability of the normal and malignant heterologous tissue, respectively.

Continuous growth of the malignant human skin tumors on the CAM could not be established. On the contrary, it was evident that the tumors grew less well than the normal tissue. Some of the differences in the viability between these two types of tissues will be discussed in this connection.

Small tissue fragments were transplanted to the 9—11-day-old membrane. As seen in the survey of the literature, the 9-day CAM has generally been used, though the age of the eggs varied from 2—16 days. The present study confirms the opinion that the 9-day membrane is most suitable at least for tissues growing relatively slowly. In membranes younger than 9 days, the graft adhered more slowly to the CAM and relatively abundant necrosis occurred in the grafts. In membranes older than 11 days, on the other hand, bleedings disturbed the development. It is obvious that the peculiar development of the blood vessels of the membrane (11) was the cause of these phenomena. The foreign tissue reactions reported by some authors, *e. g.*, M u r p h y (60) and M i n o u r a (56) were not found to occur in the older membranes.

Homologous embryonic skin tissue grew relatively well on the membrane and its viability seemed to be better than that of adult chick skin, which showed a tendency to wrinkle and perhaps for this reason was less successful as a graft. As compared with the controls, the growth of embryonic tissue grafts was found to be slower and the developed down feathers were shorter and more scarce. Remarkable in this series was the appearance of a large vesicle formation under the CAM bulging from the endoderm. It is obvious that the transplanted embryonic tissue plays a role in the development of this formation, as in spite of moderate mem-

branous reactions in most series, such a notable vesicle formation never appeared. Similar vesicle formations are mentioned by Murray (64) and Karnofsky and co-workers (45), who also used chick embryo tissue as transplantation material. Accordingly, it is not the homologous embryonic skin tissue alone that causes the reactions. There must rather be a vesicle promoting factor or substance in the homologous embryonic tissue, *per se*. The wall of the vesicle resembled the normal CAM and no tissue parts of the transplanted skin graft were found in it. The phenomenon is of interest and deserves further studies.

Embryonic human skin tissue grew relatively well on the membrane and the results were comparable with those obtained with the homologous embryonic skin. Mitoses appeared, above all, around hair follicles and in the epidermal layer, while the dermis swelled 2—2 ½ times its original size owing to the cell multiplication and edema. No foreign tissue reactions were found in the graft or membrane. The mitotic activity was more frequent in embryonic skin grafts than in the adult skin tissue, and was, in all probability, due to the difference in the basic structure of tissues. On the contrary, the retransplantation of embryonic human skin grafts to the second passage was less successful than the corresponding transplantations with the adult human skin.

Goodpasture, Douglas and Anderson (26) as well as Blank, Coriell and Scott (4) had previously reported on the good viability of the human skin on the membrane. The former workers succeeded in retransplanting the skin to the second and the latter to the fourth passage having the skin viable on the membrane for 27 days altogether. In the present study, the author succeeded in retransplanting the skin through three passages and the period of the viability of the skin was 30 days, as each passage took 10 days. After each passage, the necrosis increased in the skin grafts, and the skin tissue tended to die on the membrane. Thus the adaption of the skin tissue to the membrane for continuous retransplantation did not succeed.

In general, the series performed with human tissues on the CAM do not agree with the expectations. According to an assumption (57, 2), the failure could be due to the elapse of time between the biopsy and transplantation. This hypothesis is not supported by the observation that it was successful to cultivate the adult human skin at least after 15 days' preservation time at 5° C. Maybe it was a mere coincidence, but it was interesting to see how the refrigerated skin grafts taken from a lower limb amputated because of *thrombangitis obliterans* showed the best viability. Perhaps the cells here were accustomed to poorer living circumstances and the grafts therefore showed better viability on the CAM.

There is also another possible explanation. In this type of skin the capillary bed is increased and enlarged and offers better possibilities for nutrition. The ordinary histologic examination of the original tissue did not, however, clearly support the last-mentioned assumption.

Benign human tumors have rather seldom been cultivated on the membrane. Goodpasture, Douglas and Anderson (26) found that naevus and keloid tissues showed better viability than the normal skin, which they thought to be due to the structure of tissue promoting incorporation. Hurst, Cooke and McLennan (41) transplanted, *e. g.*, tissue from fibroadenoma of breast to the membranes of 16 duck eggs, and in 3 of them a take was found 12 days later. In the present study naevus and keloid tissues did not grow so well as skin tissues, which might be due to the fact that these tissue grafts could not be cut so thin as skin grafts. The tumors resembling the normal skin tissue in structure, such as naevus, keloid and fibroma tissues, showed, in my experiments, better viability on the membrane than those with more active cell functions (*verruca plantae pedis* and leukoplakia tissues). Perhaps the relative failure to cultivate the latter tissues on the membrane was due to the fact that their structure was unsuitable for the invasion of blood vessels into the grafts. In these original tissues, the number of inflammatory cells was also greater as compared with the others.

The cultivation results with malignant human tumors on the CAM have usually been poor and the general opinion has been negative (94, 41, 47). During the present study, two research groups have, however, published better results. In 1952, Kaufman and co-workers (48) reported on the successful cultivation of 26 tumors on the membrane. They even succeeded in retransplanting 5 of them to the second and two to the third passage. In 1956, in a more detailed paper (49), they reported the various types of tumors used, *viz.*, 7 sarcomas, 2 astrocytomas, one glioblastoma, 4 breast carcinomas, one thyroid carcinoma, one neurilemoma, 2 melanocarcinomas, and one renal cell carcinoma. Three sarcomas, one astrocytoma and the neurilemoma survived the second and one sarcoma and one astrocytoma survived the third passage.

Dagg, Karnofsky, Toolan and Roddy (10) reported on successful results with cultivation of 3 tumor types on the CAM in 1954. The tumors were induced by injecting suspended tumor mince into rats treated with cortisone (100). The tumor tissues had been maintained in the rats for several months. The tumors grew well on the membrane and could be transplanted through several passages. The earlier results obtained from "pure" human tumors by Karnofsky and co-workers (47) were, on the other hand, considerably less successful.

The transplantation results obtained from the present study with the malignant tissue agreed, in general, with those reported by previous workers, *i. e.*, by Hurst and co-workers (41), Karnofsky and his research group (47) and Sommers and his associates (91). On the other hand, the results published more recently and discussed above were better than ours. The very reason for this may be found in the types of tumors. In the present study, malignant human skin tumors were used, while Kaufman and co-workers (48, 49) obtained the best results with sarcoma and glioblastoma tissues and Dagg, Karnofsky, Toolan and Roddy (10) employed human tumor tissue first grown in rats treated with cortisone. The malignant epidermal tumors have previously been cultivated by Hurst and co-workers (41) and Sommers and his associates (91). The former reported the take on one of 5 survivors using "secondary from carcinoma of lip" and the latter reported the successful transplant yield to be about one-seventh (ca. 14 per cent) with epidermoid carcinomas from different organs.

In the present study, the successful transplant yield obtained from 54 skin carcinomas grafted to a total of 281 eggs was about 14 per cent. No clear correlation was found between the growth of the graft and the cell differentiation grade of the malignant tissue. This was also true concerning the growth of the graft and the activity of the stroma reaction in the original tissue as well as the number and quality of the inflammatory cells. If the graft was severely infected, which very seldom occurred as attempts were made to select the tissue for biopsy specimens from less necrotic lesions, the embryos died of infection in spite of the antibiotics used.

The malignant skin tissue clearly grew on the membrane more poorly than the corresponding normal skin tissue. The following hypotheses might perhaps throw light on this fact.

1) Owing to their increased activity, the malignant cells died sooner than the normal because of poor nutrition on the CAM. It has to be kept in mind, however, that embryonic skin tissue, where mitosis was more frequent than in the normal tissue, showed fairly good viability on the membrane. In addition, homologous tumors, such as Rous-sarcoma, grew on the membrane almost better than the normal skin tissue.

2) A substance indispensable to the living functions of malignant cells might be missing in embryonated chicken eggs, or the cells might be incapable of preparing it from the nutriments of the egg. It may also be possible that

3) chicken eggs might exert some kind of inhibitory reaction to the malignant tissue, for the cell reaction in the membrane had clearly in-

creased in transplanting these tissues. This reaction, however, may have been due to other reasons, which will later be discussed in more detail. The previous observation might also be recalled, *viz.*, that the chicken embryo with its membranes has no defense mechanism against foreign tissues (78, 30, 71, 80, 81, 82).

4) The malignant tissue grafts were relatively small in size. To prevent drying, they were transported to the laboratory in saline solution. It can be assumed that saline had rinsed from the tissue some essential factor needed, *e. g.*, in the period before the membranous nutrition had developed in full. The control experiments in which the malignant tissue was transplanted "fresh" to the membrane did not, however, support this assumption. It should be kept in mind that at least the refrigerated normal skin prevailed long in good condition for grafting. Accordingly, it is unlikely that a short stay (2—3 hours) in saline would have any harmful influence upon tissues.

In the last series normal and malignant skin tissues were grafted simultaneously on the same membrane. Previously, Horning and Byrne (39) cultivated *in vitro* a mouse sarcoma in various media and found that "cell proliferation is more active when the neoplastic tissue is cultivated in a medium of testicular extract from sarcomatous bearing mice than in a medium composed of testicular extract from healthy normal mice". Equally, the embryonic juice does not so readily induce cell multiplication as the tumor extract.

In the present study, the malignant skin tissue showed poorer viability than the skin grafts. Nor did the normal tissue stimulate the growth of the malignant grafts, because the cultivation results of the malignant tissue were equal to those obtained from the grafting of malignant tissue alone.

The normal skin tissue showed poorer viability in these experiments when together with the malignant tissue. The slices of the normal skin were obtained in connection with operations, as it was impossible otherwise to reserve normal skin tissue for the days when malignant tissue was available. It can thus be suspected that in some respects the grafts taken from the slices of skin would have been less viable than the previous normal ones, though no macroscopic or microscopic differences were found. Secondly, the poor viability might have been caused by the fact that the dying malignant tissue had a harmful influence on the normal skin graft. To some extent, this assumption is supported by Fig. 19, in which the degeneration of the skin graft is most marked in the edge where the malignant skin graft was placed, but no final conclusions can be drawn on the basis of this single preparation.

It was mentioned previously that the evaluation of membranous reactions and the conclusions have to be made with certain caution since the CAM reacts to different stimuli extremely easily. Some types of reaction are, however, worth mentioning. The ectoderm disappeared in 1—2 days from underneath the graft, and the remnants obviously caused the development of epithelial pearls. In the margins and folds of the grafts, the ectoderm of the CAM became hypertrophized. The endoderm also hypertrophized opposite the graft. The changes occurred in all series so that there seemed to be no difference in membranous proliferations caused by the various tissues. An exceptionally large follicular formation originating from the endoderm was met with, when transplanting the homologous embryonic skin tissue on the CAM. This phenomenon has been discussed in more detail previously.

The mesoderm usually enlarged two-to three-fold and tended to get a more fibrous structure. The enlargement was more marked in young membranes, in which the grafts sank more easily into the membrane than in the older ones. The sinking was also influenced by other factors, *e. g.*, the relation of the weight and area of the graft and the tendency of the graft to wrinkle.

Nicholas and Rudnick (65) paid attention to this phenomenon and considered that mammalian tissues had a tendency to penetrate into the membrane. Goodpasture, Douglas and Anderson (26) reported that the epidermal fibrils of the skin graft intermingled with the ectoderm of the membrane so that the skin graft remained well on the membrane. The present study supports the latter observation, as the skin grafts remained better on the membrane than the other tissues. It is difficult to say in which way the sinking influenced the viability of the graft, but one might assume that it would be more advantageous for the skin tissue to remain on the membrane, while, *e. g.*, the mammary tissue would live better inside the mesoderm. No convincing evidence can, however, be given on behalf of this assumption.

In addition to the enlargement, the accumulation of inflammatory cells occurred in the mesoderm, especially round the dead grafts. These were most abundant in the series, in which the malignant skin tissue and adult hen skin were grafted on the membrane. Since the former grafts grew poorly almost without exception, it is probable that the occurrence of inflammatory cells was rather caused by stimuli from the dying tissue than by the presence of the malignant tissue. Since the adult hen skin showed better viability, the reaction in this case might have been caused by some other factor.

Previous investigators found only one change caused by malig-

nant tissue in the chick embryo, *viz.*, general edema caused by *lymphogranulomatosis maligna* (47). In the present study, all embryos and their organs (liver, spleen and lungs) were studied both microscopically and macroscopically, but no signs of changes were found. The traumatic mortality of the embryos was equally small in both normal and malignant transplantations, and was further relatively small as compared with the number of eggs used (in total, 10 per cent). The mortality of chicken embryos due to infections was, on the other hand, greater when transplanting malignant tissues, probably because these specimens *per se* were infected more often than the normal tissue. There is no evidence that the malignant tissues would have been able to injure the embryos and thus weaken their resistance.

It seems to be very difficult to cultivate human skin carcinomas continuously on the CAM by using the general graft transplantation method or to produce any specific lesions in the embryonated egg by tumor grafting. This need not, however, mean that human cancer could not be cultivated on the membrane by some other methods. The work by Dagg, Karnofsky, Toolan and Roddy (10) supports this assumption. That the life of malignant tissue can be observed on the membrane relatively easily, facilitates the positive solution. The observation that the malignant tissue did not live so well as the normal seems to be of interest. The investigation of this problem will probably elucidate some aspects in cancer studies.

SUMMARY

The purpose of the present study was to compare the growth of different tissues, *viz.*, different forms of the skin tissue, on the membrane and thus throw further light on the possibilities that the chorio-allantoic membrane (CAM) offers to tissue culture studies.

On the CAM of 1060 embryonated chicken eggs, skin tissue grafts in size 4 × 5 × 0.5—1 mm were transplanted. The grafts originated from 6 chicken embryos, 6 chickens, 1 adult hen, and skin was obtained from 4 human embryos and 12 adult human beings. In addition, tissues from the following tumors were transplanted on the membrane: 1 keloid, 3 fibromas, 7 naevi, 4 verrucae of the sole, 5 leukoplakias, 33 basocellular carcinomas, 18 spinocellular carcinomas, 3 baso-spinocellular carcinomas, 3 anaplastic carcinomas of the skin and 3 mammary carcinomas.

1) Homologous skin tissue grew considerably well on the CAM, and 75 per cent of the grafts survived. No great differences were found in the viability between the skin tissue of chick embryo, chicken and adult hen, though it seemed likely that the last-mentioned showed the poorest viability.

2) In transplantation series on human skin, the difference between embryonic and adult skin grafts was very slight, as the former were successful in 75.7 and the latter in 74,2 per cent. The results were comparable with those obtained from the homologous skin tissue. The adult human skin survived transplantable at least 15 days' preservation period at 5° C. In passage experiments, I succeeded in retransplanting the embryonic tissue through the second passage, *i. e.*, in maintaining it on the membrane for 20 days in total. The adult human skin was retransplanted through 3 egg passages, *i. e.*, maintained on the membrane for 30 days in total. In each tissue necrosis increased, however, after every passage so that the tissues obviously died gradually and could not be cultivated on the membrane continuously.

3) Of the benign tumors, keloid, naevi and fibroma tissues showed the best viability, almost like the normal skin tissue. Verrucae and leukoplakiae showed poorer viability.

4) Malignant human skin tissue showed much poorer viability on the membrane than homologous skin tissue and normal human skin tissue. In the experiments in which tumor and skin grafts were placed side by

side on the same membrane the same phenomenon occurred. The tumor tissue could not be passaged to new eggs.

5) The homologous embryonic skin tissue caused a large follicular formation on the membrane, and relatively abundant inflammatory cell accumulations occurred round the dead grafts. Malignant tissues produced no changes in the embryos. No signs of metastases were seen. With regard to the eggs, the traumatic mortality was 10 and the infection mortality 2.4 per cent, in total.

6) Factors probably causing the poor viability of the malignant tissue on the membrane might be the following:

a) The malignant cells, because of their more active cell function, died under worse nutritional conditions sooner than the normal cells.

b) A specific nutriment necessary to the life of the malignant cells might be missing in the egg.

c) The hen egg developed a defense mechanism against the malignant tissue.

d) The malignant tissue grafts, owing to their small size, were more sensitive to technical interferences in spite of the attempts to perform the experiments in conformity with each other.

The conclusions drawn from these phenomena are discussed.

REFERENCES

1. Armstrong, M., and Ham, A.: Effects, Particularly Anaemia, Produced in Chicks by Growth in Their Yolk Sacs of Mouse Mammary Tumors. Cancer Research 1947 : 7 : 481.
2. Bender, D. H., Friedgood, C. E., and Lee, H. F.: Transplantation of Heterologous Tumors by the Intravenous Inoculation of the Chick Embryo. Cancer Research 1949 : *9* : 61.
3. Beveridge, W. I. B., and Burnet, F. M.: The Cultivation of Viruses and Rickettsiae in the Chick Embryo. Spec. Rep. Series, Med. Res. Counc. No 256. London 1946.
4. Blank, H., Coriell, L. L., and Scott, T. F. McNair: Human Skin Grafted upon the Chorio-allantois of the Chick Embryo for Virus Cultivation. Proc. Soc. Exper. Biol. & Med. 1948 : *69* : 341.
5. Brown, J. B., and Fryer, M. P.: Postmortem Homografts to Reduce Mortality in Extensive Burns. J. A. M. A. 1956 : *156* : 1163.
6. Burnet, F. M.: The Use of the Developing Egg in Virus Research. Spec. Rep. Series, Med. Res. Counc. No 220. London 1936.
7. Campbell, J. G.: The Cultivation of Tumours in the Fertile Egg, with Special Reference to Associated Ectodermal Lesions of the Chorioallantoic Membrane. Brit. J. Cancer 1949 : *3* : 72.
8. Campbell, J. G.: Spontaneous Hepatocellular and Cholangiocellular Carcinoma in the Duck. An Experimental Study. Brit. J. Cancer 1949 : *3* : 198.
9. Covell, W. P.: Intranuclear Inclusions in Brain of Chick Embryo after Inoculation of Egg with Virus of Equine Encephalomyelitis. Proc. Soc. Exper. Biol. & Med. 1934 : *32* : 51.
10. Dagg, C. P., Karnofsky, D. A., Toolan, H. W., and Roddy, J.: Serial Passage of Human Tumors in Chick Embryo: Growth Inhibition by Nitrogen Mustard. Proc. Soc. Exper. Biol. & Med. 1954 : *87* : 223.
11. Danchakoff, W.: The Position of the Respiratory Vascular Net in Allantois of the Chick. Am. J. Anat. 1917 : *21* : 407.
12. Danchakoff, W.: Cell Potentialities and Differential Factors Considered in Relation to Erythropoesis. Am. J. Anat. 1918 : *24* : 1.
13. Danchakoff, W.: Equivalence of Different Hematopoetic Anlages. Am. J. Anat. 1918 : *24* : 127.
14. Dantschakoff, W.: Wachstum transplantierter embryonaler Gewebe in der Allantois. Ztschr. f. Anat. u. Entwicklungsgesch. 1924 : *74* : 401.
15. Dantschakoff, W., and Gagarin, A.: Embryoherz in der Chorio-Allantois des Hühnchens. Ztschr. f. Anat. u. Entwicklungsgesch. 1929 : *89* : 754.
16. D'Aunoy, R., and Evans, F. L.: Histology of the Normal Chorio-allantoic Membrane of the Developing Chick Embryo. J. Path. & Bact. 1937 : *44* : 369.
17. Duran-Reynals, F.: A Hemorrhagic Disease Occurring in Chicks Inoculated with the Rous and Fuginami Viruses. Yale J. Biol. & Med. 1940 : *13* : 77.

18. Duran-Reynals, F.: A Study of Three New Duck Variants of Rous Chicken Sarcoma. Cancer Research 1947 : *7* : 99.
19. Duran-Reynals, F.: Neoplastic Infection and Cancer. Am. J. Med. 1950 : *8* : 490.
20. Duran-Reynals, F., and Bunting, H.: The Recíprocal Infection of Ducks and Chickens with Tumor-inducing Viruses. Cancer Research 1942 : *2* : 343.
21. Duthie, E. S.: Experiments on Chorio-allantoic Membrane of Chick Egg with Regard to Action of Homogeneous X-ray Irradiation. Brit. J. Radiol. 1934 : *7* : 238.
22. Duval, M.: Etudes histologiques et morphologiques sur les Annexes des Embryons d'oiseau. J. de l'Anat. et Physiol. 1884 : *20* : 201.
23. Feigenbaum, A., and Kornblueth, W.: Human Conjuctiva Grafted on the Chorioallantois of Chick Embryo. Arch. Opht. 1948 : *39* : 67.
24. Füllborn, F.: Beiträge zur Entwicklung der Allantois der Vögel. Inaugural Dissertation, Berlin 1895.
25. Goldsworthy, N. E., and Moppett, W.: The Reactions of the Chorio-allantoic Membrane of the Chick to Certain Physical and Bacterial Agents. J. Path. & Bact. 1935 : *41* : 529.
26. Goodpasture, E. W., Douglas, B., and Anderson, K.: A Study of Human Skin Grafted upon the Chorio-allantois of Chick Embryos. J. Exper. Med. 1938 : *68* : 891.
27. Goodpasture, E. W., and Anderson, K.: Immunity to Fowlpox Studied by Means of Skin Grafts on Chorio-allantois of Chick Embryo. Arch. Path. 1940 : *30* : 212.
28. Goodpasture, E. W., and Anderson, K.: Virus Infection of Human Fetal Membranes Grafted on the Chorio-allantois of Chick Embryos. Am. J. Path. 1942 : *18* : 563.
29. Goodpasture, E. W., and Anderson, K.: Infection of Human Skin, Grafted on the Chorioallantois of the Chick Embryos, with the Virus of Herpes Zoster. Am. J. Path. 1944 : *20* : 447.
30. Grasset, E.: A Comparative Study of the Aptitude of the Higher Animal Organism to Acquire Immunity throughout the Vital Cycle and the Relation of this Aptitude to the Hereditary Transmission. South African Inst. Med. Res. Pub. 1929 : *4* : 171.
31. Greene, H. S. N.: Heterologous Transplantation of Human and Other Mammalian Tumors. Science 1938 : *88* : 357.
32. Hamburger, V.: Morphogenetic and Axial Self-differentiation of Transplanted Limb Primordia of 2-day Chick Embryos. J. Exper. Zool. 1937 : *77* : 379.
33. Hancox, N. M.: The Survival of Transplanted Embryo Bone Grafted to Chorioallantoic Membrane, and Subsequent Osteogenesis. J. Physiol. 1947 : *106* : 279.
34. Heilman, F. R.: The Cultivation of Malignant Cells in the Yolk Sac of the Embryonated Egg. Proc. Staff Meet., Mayo Clin. 1943 : *18* : 223.
35. Heilman, F. R., and Bittner, J. J.: Observations on Mouse Tumors Cultivated in the Yolk Sac of the Embryonic Chick. Cancer Research 1944 : *4* : 578.

36. Hiraiwa, Y. K.: Studies on Grafts of Embryonic Tissues of the Rat on the Chorio-allantoic Membrane of the Chick. J. Exper. Zool. 1927 : *49* : 441.
37. Hirota, S.: On the Seroamniotic Connection and the Foetal Membranes in the Chick. J. of College of Science, 1894 : *6* : 337.
38. Hoadley, L.: Developmental Potencies of Parts of the Early Blastoderm of the Chick. II. The Epidermis and the Feather Primordia. J. Exper. Zool. 1926 : *43* : 179.
39. Horning, E. S., and Byrne, J. M.: On the Effect of Embryonic and Various Glandular Extracts upon the Growth of a Mouse Sarcoma Cultivated *in Vitro:* A Cytological Study. Australian J. Exper. Biol. & M. Sc. 1929 : *6* : 1.
40. Hungate, R. E., Taylor, A., and Thompson, R. C.: The Relations to Chick Tissues of Tumors Produced by the Yolk Injection Technic. Cancer Research 1944 : *4* : 289.
41. Hurst, E. W., Cooke, B., and McLennan, G. C.: A Note on Survival and Growth of Human and Rabbit Tissue (Normal and Neoplastic) on the Chorio-allantois of the Chick and Duck Embryo. Australian J. Exper. Biol. & M. Sc. 1939 : *17* : 215.
42. Huxley, J. S., and Murray, P. D. F.: A Note on the Reactions of Chick Chorio-allantois to Grafting. Anat. Rec. 1924 : *28* : 385.
43. Jacoby, F., McDonald, S., and Woodhouse, D. L.: Growth of a Dibenzanthracene-produced Mouse Sarcoma on the Chorio-allantoic Membrane of the Chick. J. Path. & Bact. 1943 : *55* : 409.
44. Julianelle, L. A.: Studies on the Infectivity of Trachoma. XII. Additional Observation on the Cultiviability of the Virus. Am. J. Ophth. 1943 : *26* : 280.
45. Karnofsky, D. A., Parisette, L. M., Patterson, P. A., and Jaques, J. A.: The Behavior and Growth of Homologous and Heterologous Normal and Neoplastic Tissues on the Chick Embryo; and the Influence of Various Agents on Tumor Growth. Acta Unio internat. contra Cancrum 1949 : *6* : 641.
46. Karnofsky, D. A., Patterson, P. A., and Ridgway, L. P.: The Growth and Histology of a Variety of Mouse Tumors Explanted to the Chorioallantoic Membrane of the Chick Embryo. Cancer Research 1950 : *10* : 228.
47. Karnofsky, D. A., Ridgway, L. P., and Patterson, P. A.: Tumor Transplantation to the Chick Embryo. Ann. New York Acad. Sc. 1952 : *55* : 313.
48. Kaufman, N., Prieto, L. C., Jr., Mason, E. J., and Kinney, T. D.: The Survival of Human Tumor Tissue on the Chorioallantoic Membrane of the Chick Embryo. Am. J. Path. 1952 : *28* : 561.
49. Kaufman, N., Kinney, T. D., Mason, E. J., and Prieto, L. C.: Maintenance of Human Neoplasm on the Chick Chorio-allantoic Membrane. Am. J. Path. 1956 : *32* : 271.
50. Keogh, E. V.: Ectodermal Lesions Produced by the Virus of Rous Sarcoma. Brit. J. Exper. Path. 1938 : *19* : 1.
51. Kirber, H. P., Kirber, M. W., and Henle, W.: Transplantation of Cornea to Chorio-allantoic Membrane, with Observations on Virus Inoculation. Proc. Soc. Exper. Biol. & Med. 1950 : *73* : 481.

52. Kiyono, K., and Sueyasu, Y.: Ueber die Implantation der Embryonalgewebe verschiedener Tierspezies in die Hühner und Entenembryone. Gann 1917 : *11* : 13.
53. Levi-Montalcini, R.: Effects of Mouse Tumor Transplantation on the Nervous System. Ann. New. York Acad. Sc. 1952 : *55* : 330.
54. Matthews, D. N.: Storage of Skin for Autogenous Grafts. Lancet 1945 : *1* : 775.
55. Milford, J. J., and Duran-Reynals, F.: Growth of a Chicken Sarcoma Virus in the Chick Embryo in the Absence of Neoplasia. Cancer Research 1943 : *3* : 578.
56. Minoura, T.: A Study of Testis and Ovary Grafts on the Hen's Egg and Their Effects on the Embryo. J. Exper. Zool. 1921 : *33* : 1.
57. Murphy, J. B.: Transplantability of Malignant Tumors to Embryos of a Foreign Species. J. A. M. A. 1912 : *59* : 874.
58. Murphy, J. B.: Transplantability of Tissues to the Embryo of Foreign Species. J. Exper. Med. 1913 : *17* : 482.
59. Murphy, J. B.: Studies in Tissue Specificity. II. J. Exper. Med. 1914 : *19* : 181.
60. Murphy, J. B.: Factors of Resistance to Heteroplastic Tissue Grafting. Studies in Tissue Specificity. III. J. Exper. Med. 1914 : *19* : 513.
61. Murphy, J. B.: The Effect of Adult Chicken Organ Grafts on the Chick Embryo. J. Exper. Med. 1916 : *24* : 1.
62. Murphy, J. B.: Heteroplastic Tissue Grafting and the Mechanism of Defence. Monogr. Rockef. Inst. Med. Research 1926 : *21* : 5.
63. Murphy, J. B., and Rous, P.: The Behaviour of Chicken Sarcoma Implanted in the Developing Embryo. J. Exper. Med. 1912 : *15* : 119.
64. Murray, P. D. F.: Chorio-allantoic Grafts of Fragments of the Two-day Chick with Special Reference to Development of Limbs, Intestine, and Skin. Australian J. Exper. Biol. & M. Sc. 1928 : *5* : 237.
65. Nicholas, J. S., and Rudnick, D.: The Development of Embryonic Rat Tissues upon the Chick Chorio-allantois. J. Exper. Zool. 1933 ; *66* : 193.
66. Oakley, C. L.: Chorio-allantoic Grafts of Liver. J. Path. & Bact. 1938 : *46* : 109.
67. Oker-Blom, N.: The Behaviour of Virus from Chicken and Duck Tumors in Embryonated Eggs of Chickens and Ducks. Acta path. et microbiol. Scandinav. 1951 : *28* : 1.
68. Oker-Blom, N., and Alfthan, O.: Transplantation of Human Normal and Neoplastic Tissue to the Chorio allantoic Membrane of Embryonated Chicken Eggs. Ann. med. exper. et biol. Fenniae 1954 : *32* : 143.
69. Oker-Blom, N., and Strandström, H.: Growth of the Egg Adapted Rous Sarcoma in Relation to the Age of the Embryo. Ann. med. exper. et biol. Fenniae. In press.
70. Pierce, M.: Cultivation of Human Leukemic Leukocytes on Chorioallantoic Membrane of Chicken Egg. Arch. Path. 1942 : *34* : 538.
71. Polk, A., Buddingh, G. J., and Goodpasture, E. W.: An Experimental Study of Complement and Hemolytic Amboceptor Introduced into Chick Embryos. Am. J. Path. 1938 : *14* : 71.
72. Rawles, M. E.: Transplantation of Normal Embryonic Tissues. Ann. New York Acad. Sc. 1952 : *55* : 302.

73. Romanoff, A. L.: Membrane Growth and Function. Ann. New York Acad. Sc. 1955 : *55* : 288.
74. Romanoff, A. L., and Romanoff, A. J.: The Avian Egg. New York 1949.
75. Rothbard, S., and Herman, J. R.: Attempts to Propagate Fowl Tumors Produced by Benzpyrene and by Virus. Arch. Path. 1939 : *28* : 212.
76. Rous, P., and Murphy, J. B.: Tumor Implantations in the Developing Embryo. J. A. M. A. 1911 : *56* : 741.
77. Rubovits, F. E., and Abrams, B. S.: Human Ovarian Grafts onto the Chorio-allantoic Membrane of Developing Chick Embryos. Proc. Soc. Exper. Biol. & Med. 1951 : *77* : 447.
78. Rywosch, M.: Ueber Hämolyse und Baktericidie des embryonalen Hühnerblutes. Centralbl. f. Bakt. I Abt., Orig. 1907 : *44* : 468.
79. de Ruyck, R.: Isolement d'un virus filtrable dans une mole hydatiforme en voie de transformation chorion-epithelontense. Bull. Assoc. franc. étude cancer 1951 : *38* : 52.
80. Sandström, C. J.: Heteroplastic Transplants of Duck Kidney Tissues upon the Chick Chorio-allantois. Anat. Rec. 1932 : *52* : 69.
81. Sandström, C. J.: The Growth and Differentiation of Duck Kidney Tissue on the Chorio-allantoic Membrane of the Chick and Duck. Physiol. Zool. 1932 : *5* : 354.
82. Sandström, C. J.: Additional Observations on Heteroplastic Chorio-allantoic Grafts of Embryonic Duck Kidney Tissue. Physiol. Zool. 1934 : *7* : 279.
83. Sandström, C. J., and Kauer, G. L.: Heteroplastic Transplants of Duck Cartilage to the Chorio-allantoic Membrane of the Chick. Anat. Rec. 1933 : *57* : 119.
84. Sandström, C. J., and Kauer, J. T.: The Growth and Differentiation of Macerated Embryonic Duck Kidney Tissue on the Chorio-allantoic Membrane of the Chick. Anat. Rec. 1933 : *57* : 105.
85. Schechtman, A. M., Cohen, M. J., and Berkowitz, E. C.: Culture of Brown-Pearce Carcinoma in the Embryonated Egg. Proc. Soc. Exper. Biol. & Med. 1950 : *74* : 784.
86. Schrek, R., and Avery, R. C.: Histological Observations on Transplantable Rat and Rabbit Tumors Cultivated in the Chorio-allantoic Membrane of Chick Embryos, with Special Reference to the Walker Rat Tumor 256. Am. J. Path. 1937 : *13* : 45.
87. Schwartz, G. E.: Heteroplastic Transplantations of Variously Aged Intact and Macerated Kidney Tissue of the Rat to the Chorio-allantoic Membrane of the Chick. J. Exper. Zool. 1947 : *103* : 335.
88. Sezer, N.: Cultivation of Conjunctival and Corneal Tissue on the Chorio-allantoic Membrane. Arch. Ophth. 1950 : *44* : 703.
89. Sigel, M. M.: Influence of Age on Susceptibility to Virus Infections with Particular Reference to Laboratory Animals. Ann. Rev. Microbiol. 1952 : *6* : 247.
90. Smith, W.: Cultivation of the Virus of Influenza. Brit. J. Exper. Path. 1935 : *16* : 508.
91. Sommers, S. C., Sullivan, B. A., and Warren, S.: Heterotransplantation of Human Cancer. III. Chorioallantoic Membranes of Embryonated Eggs. Cancer Research 1952 : *12* : 915.

92. Stevenson, H. N.: The Growth of Heterologous Tumors in the Chick Embryo. Proc. New York Path. Soc. 1916 : *16* : 60.
93. Stevenson, H. N.: Tumor Immunity in the Chick Embryo. J. Cancer Res. 1917 : *2* : 245.
94. Stevenson, H. N.: Growth of Tumors in the Chick Embryo. J. Cancer Res. 1918 : *3* : 63.
95. Taylor, A., Thacker, J., and Pennington, D.: Growth of Cancer Tissue in the Yolk Sac of the Chick Embryo. Science 1942 : *96* : 342.
96. Taylor, A., Hungate, R. E., and Taylor, D. R.: Yolk Sac Cultivation of Tumors. Cancer Research 1943 : *3* : 537.
97. Taylor, A., Carmichael, N., and Norris, T.: Temperature Level and the Growth of Embryo and Tumor of Tumor-bearing Eggs. Proc. Soc. Exper. Biol. & Med. 1947 : *66* : 165.
98. Taylor, A., and Carmichael, N.: The Effect on the Embryo of Continued Serial Tumor Transplantation in the Yolk Sac. Cancer Research 1949 : *9* : 498.
99. Thiersch, J. B.: Attempts to Transmit Leucaemia of Man and of Mice to the Chick Embryo and to the Young Chick by the Amniotic and Intravenous Routes. Australian J. Exper. Biol. & M. Sc. 1944 : *22* : 57.
100. Toolan, H. W.: Transplantable Human Neoplasm Maintained in Cortisone Treated Laboratory Animals. Cancer Research 1954 : *14* : 660.
101. Twombly, G. H., and Meisel, D.: Growth of Mammalian Tumors in Fertile Eggs. Cancer Research 1946 : *6* : 82.
102. Waterman, A. J.: Heteroplastic Transplantation of Embryonic Tissues of Rabbit and Rat. Am. J. Anat. 1936 : *60* : 1.
103. Willier, B. H.: The Endocrine Glands and the Development of the Chick. Am. J. Anat. 1924 : *33* : 67.
104. Willier, B. H.: The Specificity of Sex of Organization and of Differentiation of Embryonic Chick Gonads as Shown by Grafting Experiments. J. Exper. Zool. 1927 : *46* : 409.
105. Willier, B. H.: A Study of the Origin and Differentiation of the Suprarenal Gland in the Chick Embryo by Chorio-allantoic Grafting. Physiol. Zool. 1930 : *3* : 201.
106. Willier, B. H.: Potencies of the Gonad-forming Area in the Chick as Tested in Chorio-allantoic Grafts. Arch. f. Entw. mech. 1933 : *130* : 616.
107. Willier, B. H., and Yuh, E. C.: The Problem of Sex Differentiation in the Chick Embryo with Reference to the Effects of Gonad and Non-gonad Grafts. J. Exper. Zool. 1928 : *52* : 65.

FIGURES

Fig. 1. — Skin tissue of the 10-day-old chick embryo transplanted to the 9-day-old CAM. The period of time on the membrane is 24 hours. The dermal papillae of the graft begin to develop. The dermal layer is edematic. The ectoderm of the membrane shows signs of degeneration. × 75.

Fig. 2 a. — Skin tissue of the 10-day-old chick embryo transplanted to the 9-day-old CAM. The period of time on the membrane is 5 days. The down feathers have developed considerably. × 75.

Fig. 2 b. — The control preparation. Skin tissue of the 15-day-old chick embryo. × 75.

Fig. 3. — Skin of the 10-day-old chick embryo transplanted to the 9-day-old CAM. The period of time on the membrane is 9 days. The epidermis has developed and tends to fold. Abundant keratin occurs in the folds and desquamation on the epidermis. Down papillae are spread inside the membrane. The dermal layer has turned fibrinous and now resembles that of the adult hen more than before. Well developed fat tissue can be seen in the margin of the graft and the membrane, in the middle of the figure. × 100.

Fig. 4. — Skin tissue of the adult hen transplanted to the 9-day-old CAM. The period of time on the membrane is 9 days. The graft has sunk inside the membrane. Both the epidermal and the dermal layer are well preserved, but the graft and the surrounding mesoderm are infiltrated by inflammatory cells. × 75.

Fig. 5 a. — Skin tissue of the 2-month-old human embryo transplanted to the 9-day-old CAM. The period of time on the membrane is 9 days. The dermal layer has swollen 2—3 times from its original size and has developed a convex formation on the membrane, half of which can be seen in the figure. In the margin of the membrane and the graft there appear relatively many cysts resembling epithelial pearls, which have obviously developed from the remnants of the membranous ectoderm. × 75.

Fig. 5 b. — The control preparation. Skin tissue of the 2-month-old human embryo. × 180.

Fig. 6 a. — Skin tissue of the 3-month-old human embryo transplanted to the 11-day-old CAM. No demarcation line between the dermal layer and the mesoderm of the membrane can be seen. An extremely thin layer, *stratum corneum*, developed from dead cells is noted on the peridermis. × 100.

Fig. 6 b. — The control preparation taken from the skin tissue of the 3-month-old human embryo. × 75.

Fig. 7. — Skin tissue of the 4-month-old human embryo transplanted to the 11-day-old CAM. The period of time on the membrane is 7 days. The dermal layer is so swollen that the graft has developed a convex formation on the membrane. The blood vessels of the graft are filled with the blood cells of the chick embryo. The *stratum corneum* layer is on the peridermis. The mesoderm of the membrane has become more uniform than normal. × 40.

Fig. 8 a. — Skin tissue of the 5-month-old human embryo transplanted to the 11-day-old CAM. The period of time on the membrane is 3 days. Both the epidermal and the dermal layers are much more developed than in earlier tissues. No demarcation line between the graft and the membrane can be seen. The blood vessels of the membrane begin to penetrate into the graft. × 70.

Fig. 8 b. — The control preparation. Skin tissue of the 5-month-old human embryo. × 70.

Fig. 9. — Skin tissue of the 5-month-old human embryo transplanted to the 9-day-old CAM, *the second passage*. The total period of time on the membrane is 20 days. The graft is infiltrated by rather an abundant inflammatory cell accumulation. Giant cells can be seen here and there. Both the epidermal and the dermal layers can still be distinguished, though the majority of the cells is dead. × 70.

Fig. 10. — Adult human skin tissue transplanted to the 9-day-old CAM. The period of time on the membrane is 5 days. No inflammatory cell formation or demarcation line between the graft and the membrane can be seen. The blood vessels of the membrane have interlaced with the connective tissue fibers of the corium. × 125.

Fig. 11. — Adult human skin tissue transplanted to the 11-day-old CAM, *the second passage*. The total period of time on the membrane is 16 days. The graft with folded margins has sunk into the membrane. The *stratum corneum* layer is thick and, correspondingly, the living cellular part of the epidermis has become thinner. Signs of degeneration can also be seen in the connective tissue fibers of the corium. × 70.

Fig. 12 a. — Adult human skin transplanted to the 10-day-old CAM. The period of time on the membrane is 8 days. The skin grafts were taken from the lower limb amputated because of the gangrene caused by *thrombangitis obliterans*. Before the transplantation to the CAM the skin tissue was preserved at 5° C for 15 days. Both the epidermis and the corium tissue show very good viability. No inflammatory cell accumulation can be observed. × 70.

Fig. 12 b. — Larger magnification. In the lower part of the picture a mitosis can be seen. × 950.

Fig. 13 a. — Human naevus tissue transplanted to the 9-day-old CAM. The period of time on the membrane is 3 days. The epidermis and corium tissue show good viability on the membrane. Signs of degeneration in naevus cells can be seen. The naevus cells, being partly necrosed, do not stain easily. Free pigment occurs in the tissues. × 75.

Fig. 13 b. — Larger magnification. × 140.

Fig. 14. — Human *verruca plantae pedis* tissue transplanted to the 10-day-old CAM. The membranous mesoderm has enlarged, and the verruca tissue graft, in which the connective tissue and part of the epidermis live fairly well, has partly sunk into it. Keratinized epithelium can be seen. No clear signs of viral infections are found in the membrane. × 60.

Fig. 15 a. — Human basocellular carcinoma tissue transplanted to the 9-day-old CAM. The period of time on the membrane is 10 days. The graft has sunk into the membrane. In the middle of the picture is a long bulk in which there are basocellular carcinoma cells showing good viability. In the lower left hand corner of this bulk a mitosis can be seen. × 120.

Fig. 15 b. — Larger magnification. × 500.

Fig. 15 c. — The control preparation. Original basocellular tissue. × 300.

Fig. 16 a. — Human basocellular carcinoma tissue transplanted to the 10-day-old CAM. The period of time on the membrane is 7 days. The graft is almost entirely dead and turned to a homogeneous mass on the membrane. A viable sebaceous gland conglomeration has sunk into the mesoderm. A mesodermal formation in the lower right hand corner is an accumulation developed from giant cells, possibly still living basocellular carcinoma cells, and inflammatory cells of the membrane. × 60.

Fig. 16 b. — A larger magnification of the accumulation formed by giant cells, possibly still living basocellular carcinoma cells and inflammatory cells of the membrane. × 200.

Fig. 17 a. — Human spinocellular carcinoma tissue transplanted to the 9-day-old CAM. The period of time on the membrane is 8 days. A relatively large ball formed by atypical keratin cells is found inside the membrane. × 500.

Fig. 17 b. — The control preparation. Original spinocellular tissue. × 300.

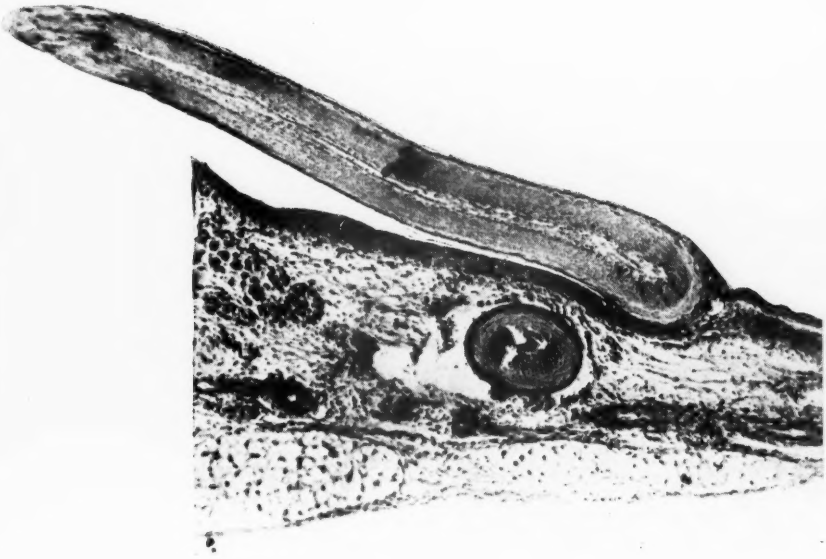
Fig. 18. — Human spinocellular carcinoma tissue and parallel normal skin tissue transplanted to the 11-day-old CAM. The period of time on the membrane is 7 days. The grafts are seen from the chorio-allantoic cavity. The carcinoma tissue graft on the left has shrunk one-third as compared with the skin graft. × 8.

Fig. 19. — Human spinocellular carcinoma tissue and parallel normal skin tissue transplanted to the 11-day-old CAM. The short arrow indicates the margins of the malignant and the normal skin tissue. The long arrows indicate the most damaged normal skin tissue situating close to the malignant tissue. × 65.

FIGURES

Fig. 1.

Fig. 2 a.



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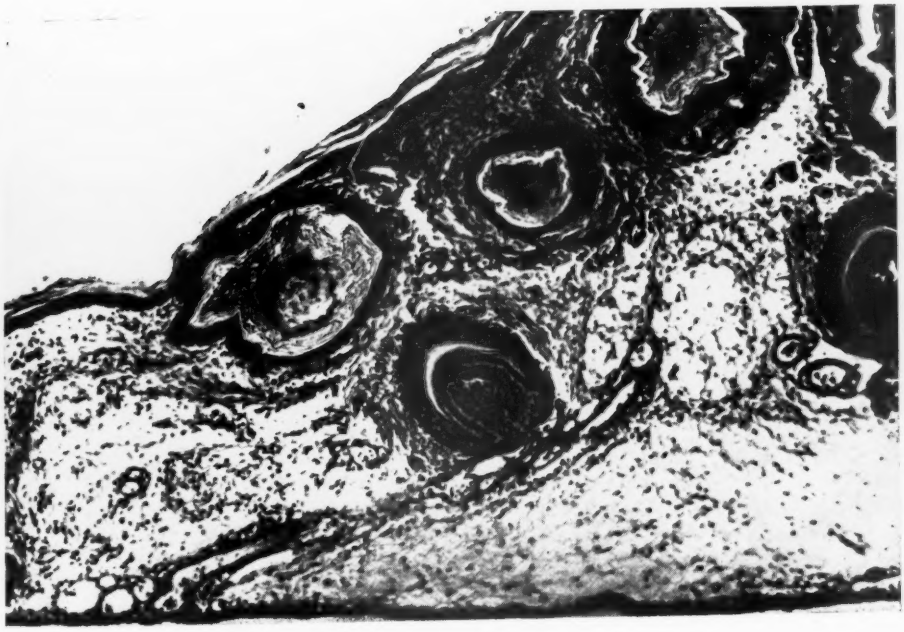
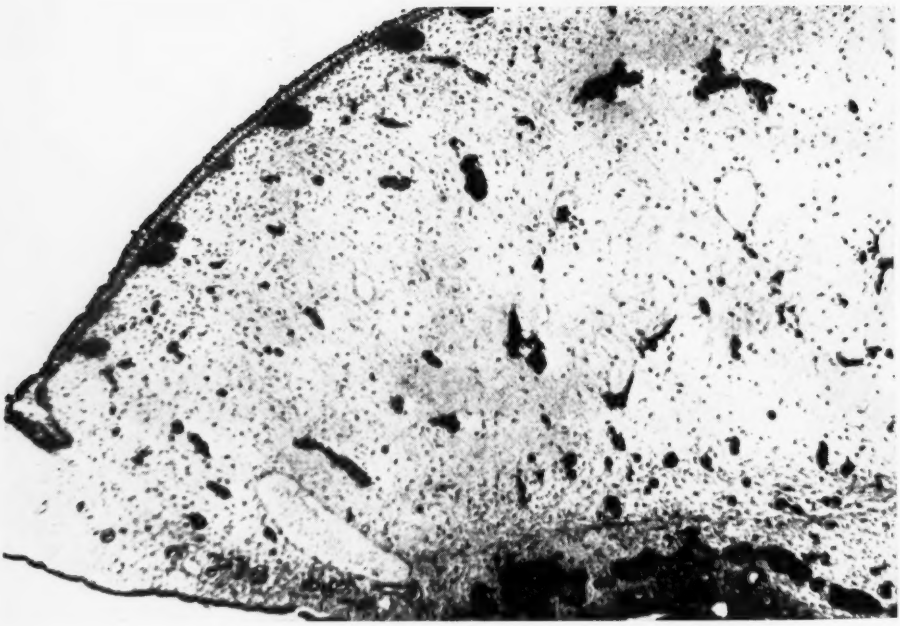


Fig. 3.

Fig. 4.



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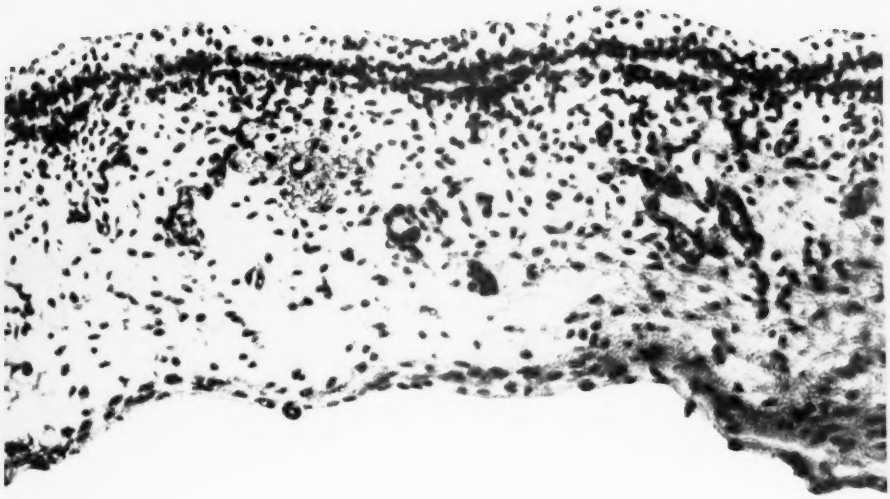

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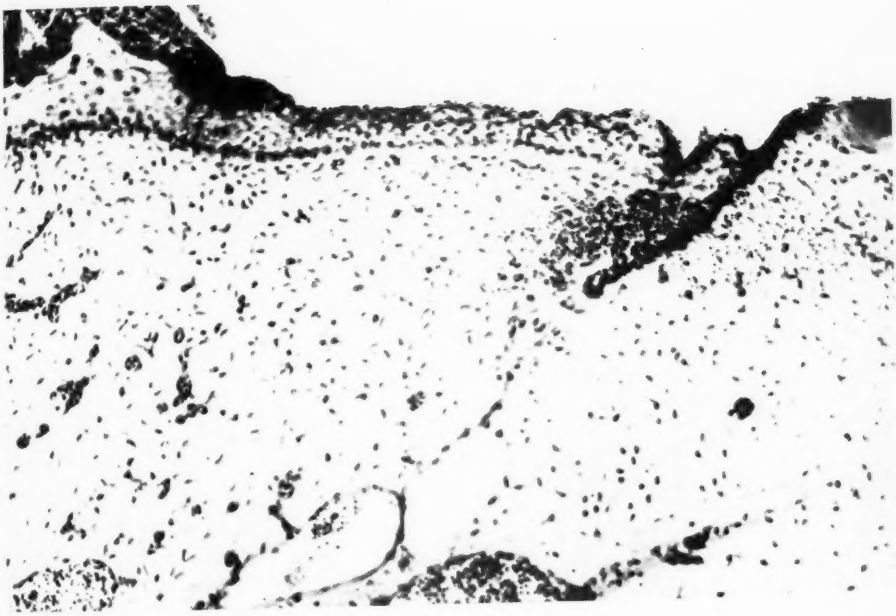

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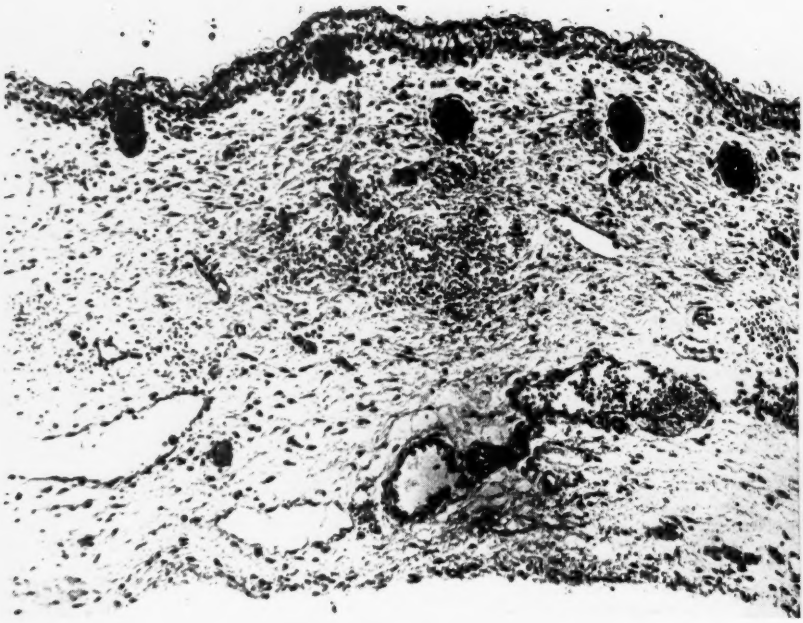
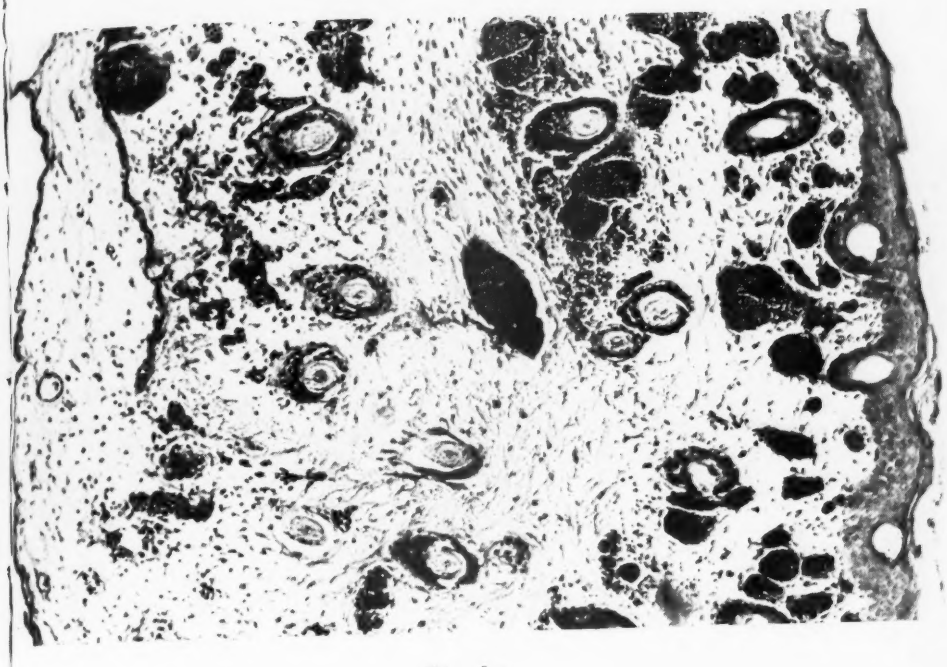


Fig. 6 b.

Fig. 7.



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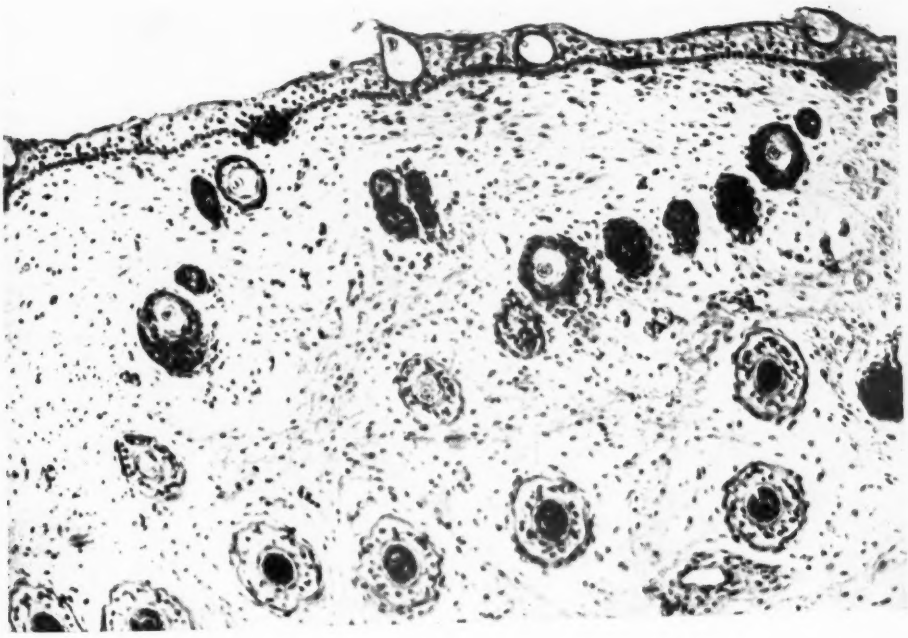
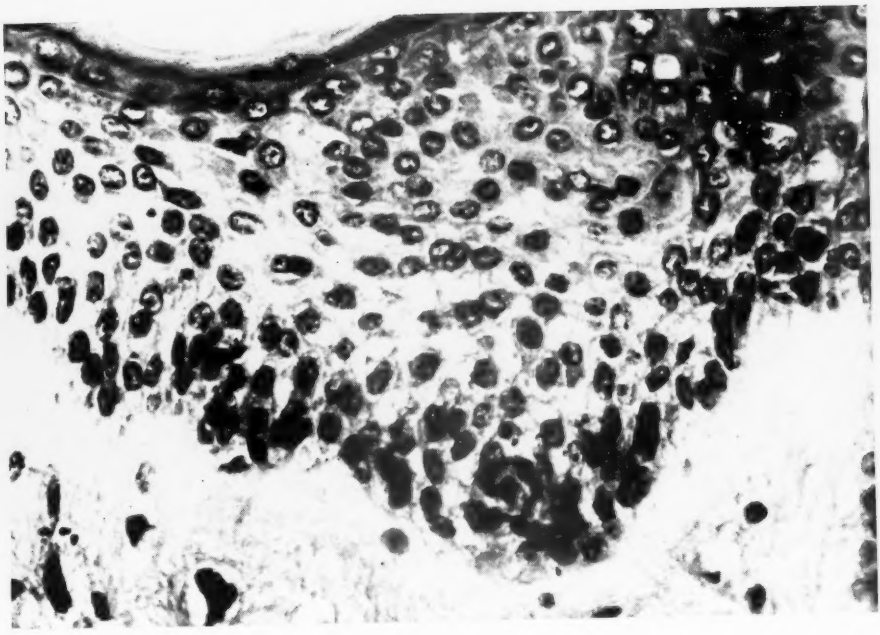


Fig. 8 b.

Fig. 9.

Fig. 10.

Fig. 11.



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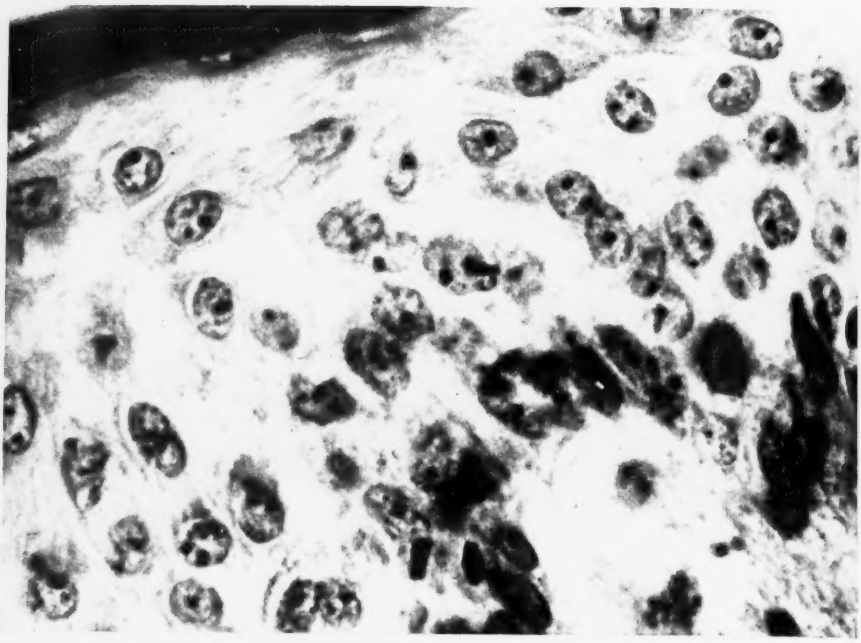

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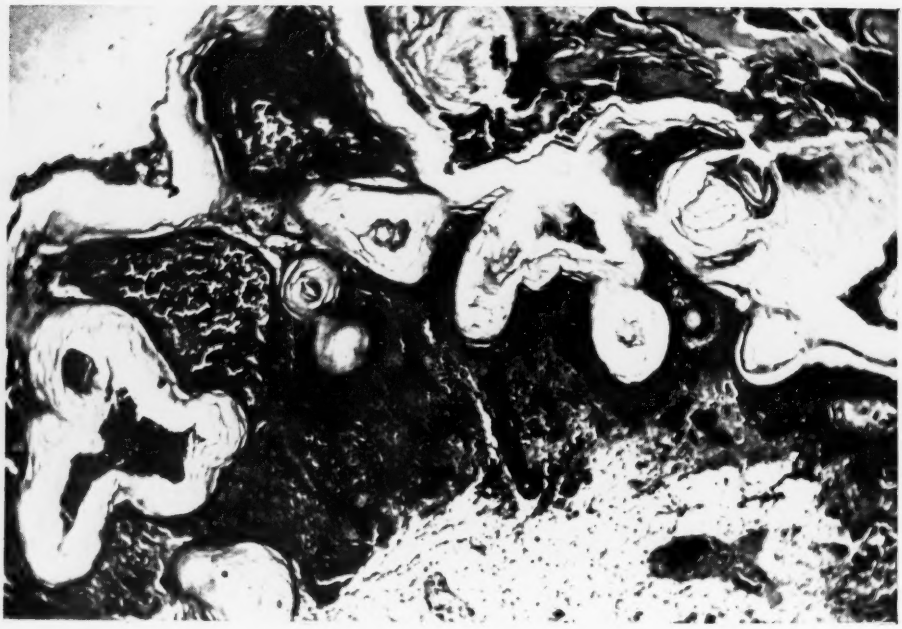
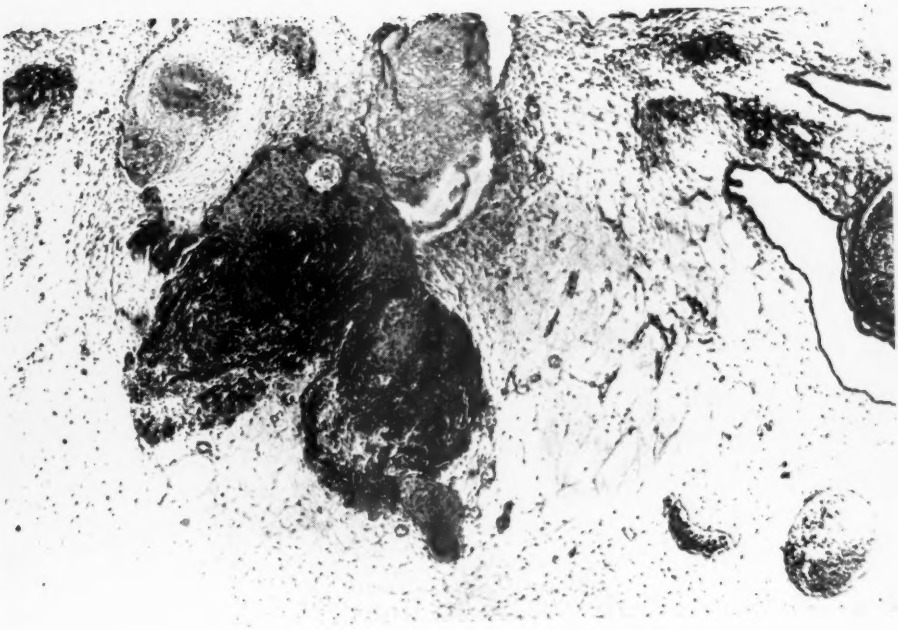


Fig. 13 a.

Fig. 13 b.



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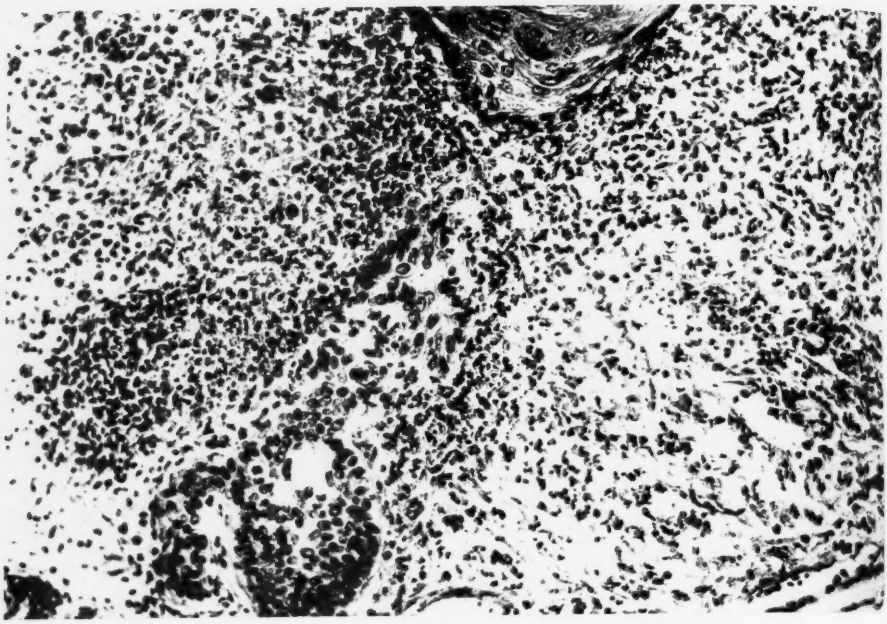

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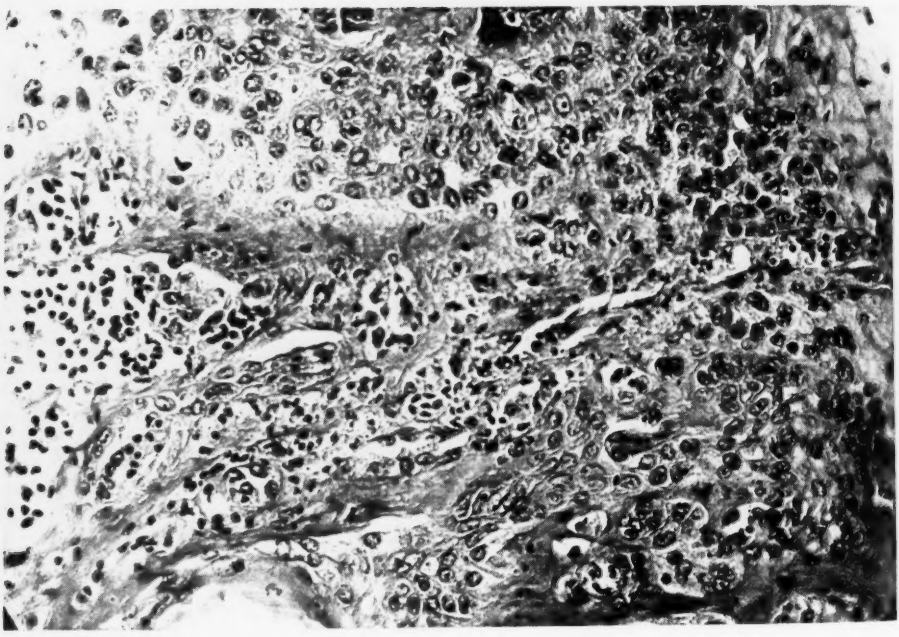

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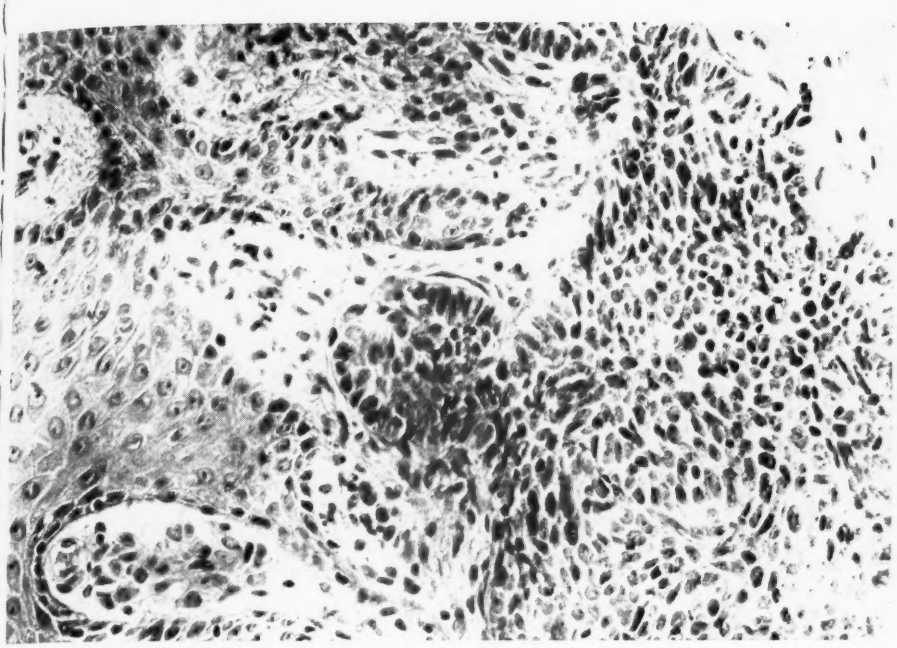

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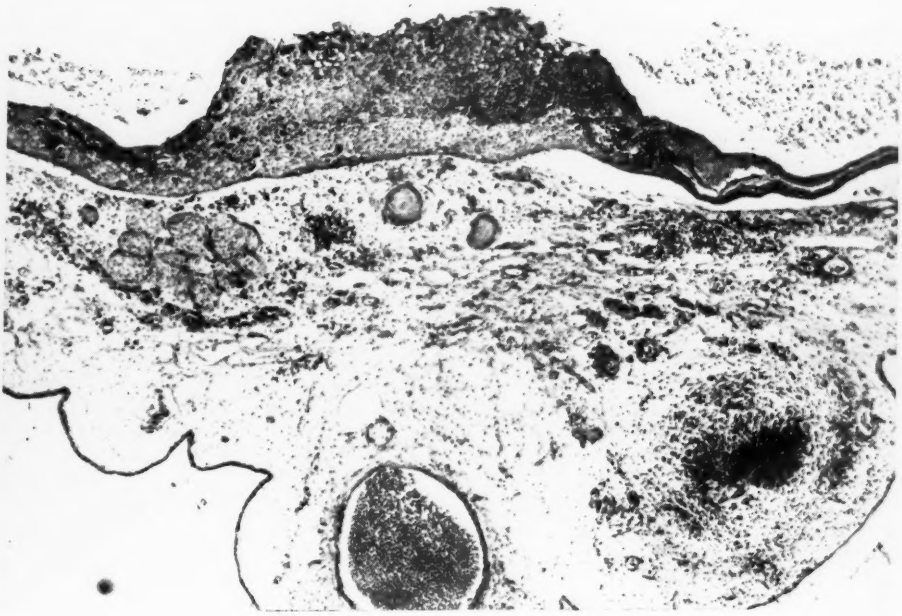

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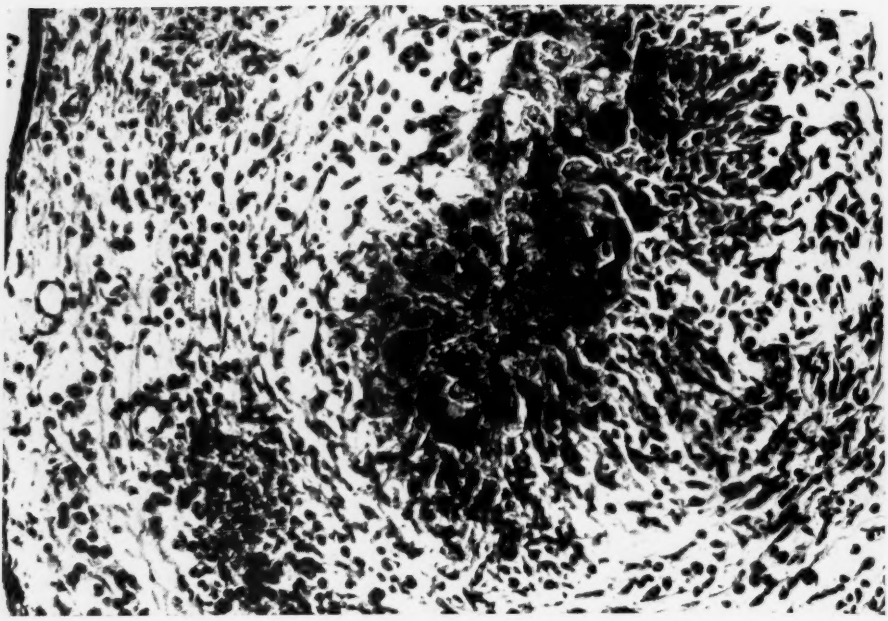

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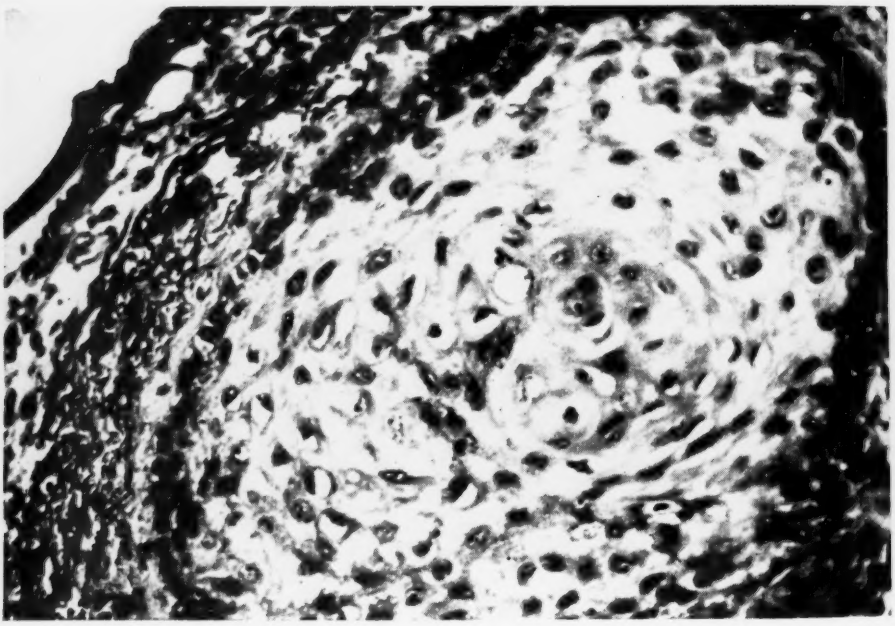

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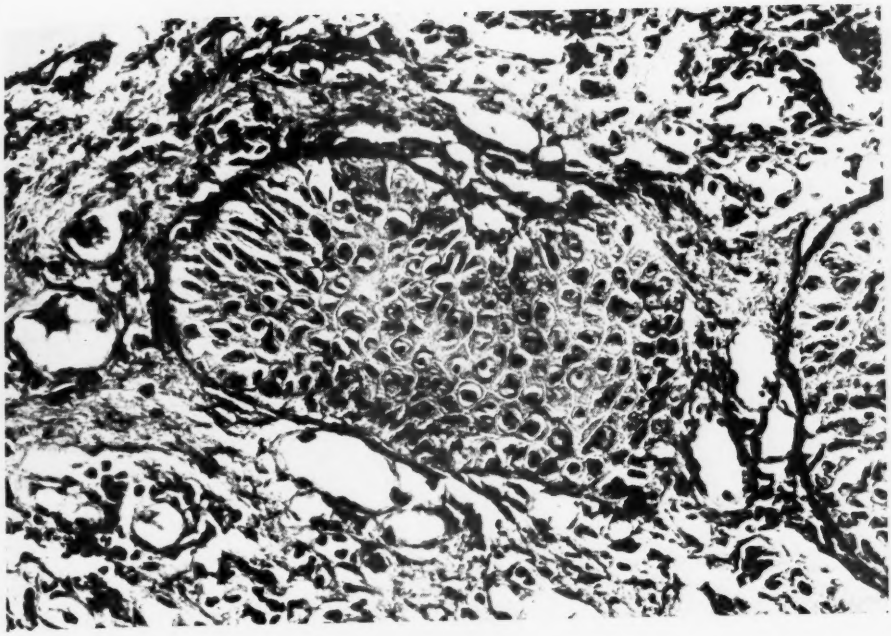

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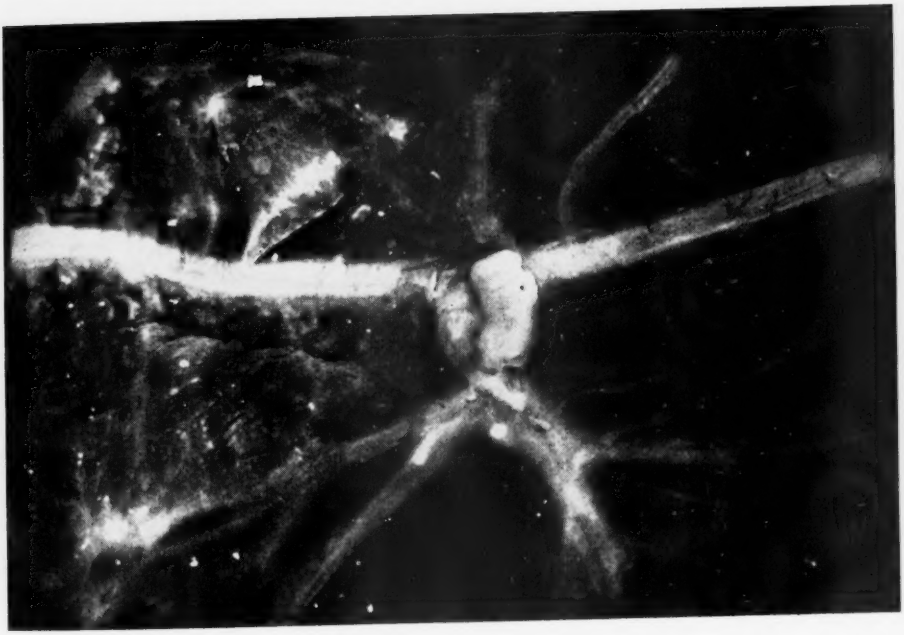

Fig. 18.

Fig. 19.

ERRATA

On page 15, line 39, fulfill should be fulfil
On page 19, line 30, 39 should be 43
On page 19, line 31, 1 spinocellular carcinoma should be 11 spinocellular carcinomas
On page 19, line 33, add after 3 carcinomas of the stomach: 9 carcinomas of the breast
On page 25, line 36, embryo should be CAM
On page 29, line 3, was similar should be was almost similar
On page 33, line 32, 53 should be 52
On page 45, line 24, tissue should be skin
On page 48, line 34, 82 should be 74.2
On page 54, line 33, necrotic should be necrotic, normal should be normal